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Original Article

ANTIDIABETIC ACTIVITY OF NOVEL CHROMENE COMPOUND ISOLATED FROM *PEPEROMIA PELLUCIDA* L. KUNTH AND *IN SILICO* STUDY AGAINST DPP-IV, ALPHA-GLUCOSIDASE, ALPHA-AMYLASE, AND ALDOSE REDUCTASE FOR BLOOD GLUCOSE HOMEOSTASIS

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ABSTRACT

Objective: During the outbreak of COVID-19, diabetes mellitus (DM) and cardiovascular disease (CVD) become risk factors for severe adverse clinical outcomes in COVID-19 patients. DM is a complex metabolic disease originating from a process of requiring adequate insulin or due to insulin resistance. This *in silico* study reveals the molecular interaction of Peperochromene A ((*S*)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one), a novel chromene compound isolated from *Peperomia pellucida* with four proteins involved in the homeostasis of blood glucose, namely dipeptidylpeptidase-IV (DPP-IV), α-glucosidase, α-amylase, and aldose reductase.

Methods: Molecular docking simulation of the ligands was performed by employing AutoDock 4.2 embedded in LigandScout at a certain position determined automatically by the program. The default parameters of the automatic settings were used to set the genetic algorithm parameters.

Results: Peperochromene A could interact with all four targets; however, it binds to alpha-glucosidase and α-amylase with Ki (inhibition constant) value better than that of acarbose, the enzymes' known inhibitor. This chromene compound also reveals an inhibition constant to aldose reductase similar with that of the enzyme inhibitor.

Conclusion: The chromene isolated from *Peperomia pellucida* is the potential to be developed as an inhibitor of the proteins involved in the homeostasis of blood glucose; thus, it can be further explored for its antidiabetic activity.

Keywords: Chromene, Diabetes mellitus, Dipeptidyl peptidase-IV, Aldose reductase, Glucose homeostasis

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INTRODUCTION

During the outbreak of COVID-19, diabetes mellitus (DM) and cardiovascular disease (CVD) become risk factors for severe adverse clinical outcomes in COVID-19 patients. DM is a complex metabolic disease originating from a process of requiring adequate insulin or due to insulin resistance. Eight major causes of hyperglycemia through the pathogenesis of type 2 DM are (1) a decrease of insulin secretion due to the dysfunction of pancreatic β-cells, (2) a decrease of incretin effect by the intestine, (3) a decrease of glucose uptake by the muscles, (4) an increase of glucagon secretion by the pancreatic α-cells, (5) an increase of lipolysis in the fat cells, (6) an increase of glucose reabsorption by the kidney, (7) an increase of hepatic glucose production, and (8) dysfunction of neurotransmitter in the brain [1, 2].

DPP-IV is a pleiotropic enzyme that inactivates two incretin hormones, e. g., glucagonlike peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These intestinal hormones have a potent insulin-secretory activity and consequent reduction of the prandial plasma glucose. Despite that, the degradation of GLP-1 and GIP by DPP-IV is very fast and governs the production of metabolites that block insulin-releasing activity. Consequently, prohibiting the breakdown of the incretin hormones, developed an innovative therapy for type 2 DM [3]. DPP-IV inhibitors have been known to bind in the amido-terminus of the substrate peptide by interacting with Glu205 and Glu206 in the S2 subsite. It was reported that the amino group of sitagliptin, a known DPP-IV inhibitor, forms 4 hydrogen bonds (HBs), one with Tyr662 and 3 HBs with Glu205 and Glu206, thus preventing the degradation of GLP-1[4].

Moreover, the aldose reductase (or aldehyde reductase, AR), an enzyme that belongs to the aldo-keto reductase family, reversibly reacts with NADPH when it reduces the substrate, glucose, or galactose, to their corresponding alcohol, sorbitol, or galactitol, respectively [5]. The abnormal increase level of blood glucose leads to its transfer into the AR-dependent polyol pathway, which consequently drains NADPH and reduces GSH level. Oxidative stress is provoked during the production of fructose from sorbitol led by sorbitol dehydrogenase. The flux of glucose via the polyol pathway gives rise to the origination of reactive oxygen species [6].

Antidiabetic drugs that work by inhibiting α-glucosidase delay dietary carbohydrate absorption and reduce both postprandial hyperglycemia and hyperinsulinemia [7]. Interestingly, a randomized, double-blind, placebo-controlled trial had reported that acarbose did not reduce the risk of major adverse cardiovascular events; nevertheless, this α-glucosidase inhibitor decreased the incidence of diabetes in Chinese diabetic patients with coronary heart disease [8].

Chromenes have been reported for their strong inhibitory activity on α-glucosidase by *in vitro* and *in vivo* studies [9]. Moreover, novel-designed chromene analogs inhibited DPP-IV in the range of 26.7–66.9% at 100 μM concentration. These compounds bind to the active site of DPP-IV by forming hydrogen bonds with Glu205, Glu206, Lys122, and Tyr238 [10]. Our previous work had successfully isolated a bioactive chromene compound, namely Peperochromene A or (S)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one (fig. 1) and Peperochromene B (8,9-dimethoxy ellagic acid) by isolation guided-activity from the herbs of *Peperomia pellucida* (Piperaceae) [11, 12].

Peperomia pellucida (L.) Kunth. belongs to the Piperaceae family and is an annual herb known as sasaladaan, sirih bumi, suruhan in Indonesia, and also known by common names pepper elder, shining

bush, and man to man. This medicinal herb is edible, both cooked or raw material, and mostly grown as ornamental foliage [13].

Several studies have reported it is known that *Peperomia pellucida* exhibits significant hypoglycemic activity and have the potential to be developed as an antidiabetic agent [12, 14, 15]. This present work aimed to predict the antidiabetic activity peperochromene A against DPP-IV, alpha-glucosidase, alpha-amylase, and aldose reductase by *in silico* study.

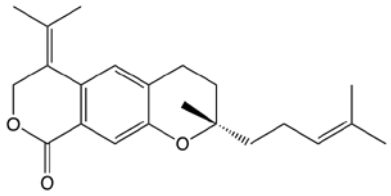

Fig. 1: 2D structure of Peperochromene A ((S)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one)

MATERIALS AND METHODS

Hardware and software. The hardware used was MacBook Pro (13-inch, M1, 2020) embedded with macOS Monterey, Apple M1 chip processor, and a memory capacity of 8 GB. Software used was MarvinSketch 17.11.0 (Academic License), LigandScout 4.1.4 (Universitas Padjadjaran License), AutoDock 4.2 embedded in the LigandScout, MacPyMOL: PyMOL 1.7.4.5 Edu.

Macromolecule preparation. The macromolecules were 3D X-ray crystallographic of

1. DPP-IV (PDB ID 4PNZ, resolution 1.90 Å in complex with omarigliptin (a long-lasting inhibitor), deposited by Scapin and Yan, downloaded from https://www.rcsb.org/structure/4PNZ) [16];

2. α-glucosidase (PDB ID 3W37, resolution 1.70 Å in complex with acarbose inhibitor, deposited by Tagami *et al.* [17] downloaded from https://www.rcsb.org/structure/3W37);

3. α-amylase (PDB ID 2QV4, resolution 1.97 Å in complex with acarbose inhibitor, deposited by Williams *et al.* [18] downloaded from https://www.rcsb.org/structure/2QV4);

4. aldose reductase (PDB ID 1Z8A, resolution 0.95 Å in complex with sulfonyl-pyridazinone inhibitor, deposited by Steuber *et al.* [19] downloaded from https://www.rcsb.org/structure/1Z8A)

Validation of the docking simulation

The inhibitors were separated from the macromolecules and validation was carried out by re-docking the inhibitors into their original position in the active pocket of the macromolecules. The grid box position and size of the active pocket were determined automatically by the LigandScout based on the original position of the inhibitors. The confirmation of the re-docked inhibitors was then superimposed with the original conformation of the inhibitors and the root mean square deviation (RMSD) of the structures was measured.

Ligand preparation

The ligand Peperochromene A, depicted in (fig. 1) was drawn in 2D structure using the MarvinSketch and converted into 3D structures by applying MMFF94 geometry optimization at default settings in the LigandScout, then the physicochemical properties (Lipinski Ro5) were predicted.

Molecular docking simulation

Molecular docking simulation of the ligands was performed by employing AutoDock 4.2 embedded in LigandScout at a certain position determined automatically by the program. The default parameters of the automatic settings were used to set the genetic algorithm parameters. The docked conformation with the highest docking score (Ei) was selected to analyze the binding mode.

RESULTS

Macromolecule preparation

DPP-IV

A 3D X-ray crystal structure of DPP-IV was employed in this work (PDB ID 4PNZ, resolution 1.90 Å, deposited by Scapin and Yan [16]. The inhibitor, omarigliptin, was co-crystallized in this enzyme and interacts by building 2 hydrogen bonds with Glu205 and Tyr662 residues (fig. 2).

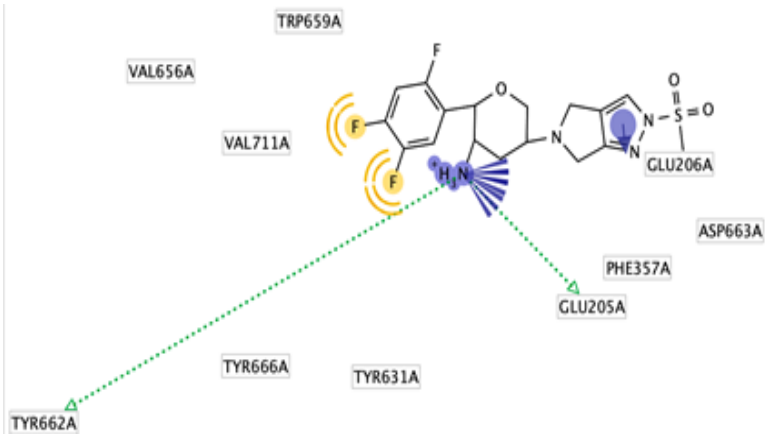

Fig. 2: The binding mode of omarigliptin with DPP-IV. Hydrogen bond donors are shown as green dashed-lines arrows. The blue highlight indicates a positive ionizable area. The yellow highlight indicates hydrophobic interaction. The blue circle with the arrow indicates aromatic stacking

α-glucosidase

A 3D X-ray crystal structure of α-glucosidase was employed in this work (PDB ID 3W37, resolution 1.70 Å in complex with acarbose inhibitor, deposited by Tagami *et al.* [17]). The inhibitor, acarbose, was co-crystallized in this enzyme and interacts by building 11 hydrogen bonds (fig. 3).

α-amylase

A 3D X-ray crystal structure of α-amylase was employed in this work (PDB ID 2QV4, resolution 1.97 Å in complex with acarbose inhibitor, deposited by Williams *et al.* [18]). The inhibitor, acarbose, was co-crystallized in this enzyme and interacts by building 10 hydrogen bonds (fig. 4).

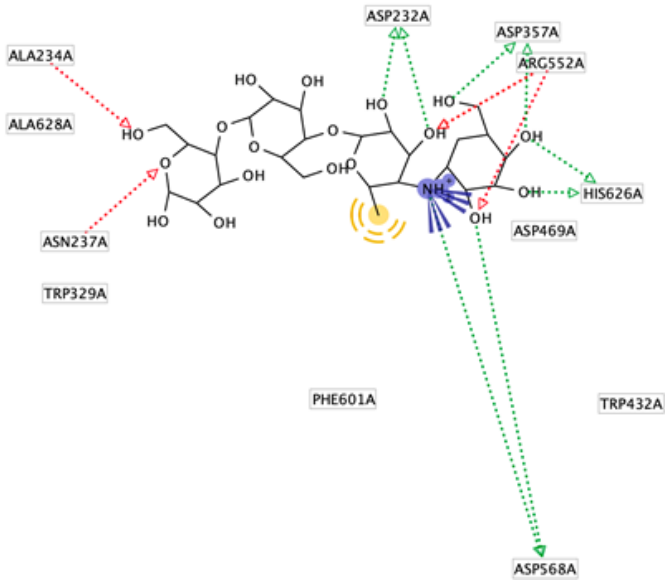

Fig. 3: The binding mode of acarbose with α-glucosidase. Hydrogen bond donors are shown as green dashed-lines arrows. Hydrogen bond acceptors are shown as red dashed-line arrows. The blue highlight indicates a positive ionizable area. The yellow highlight indicates hydrophobic interaction

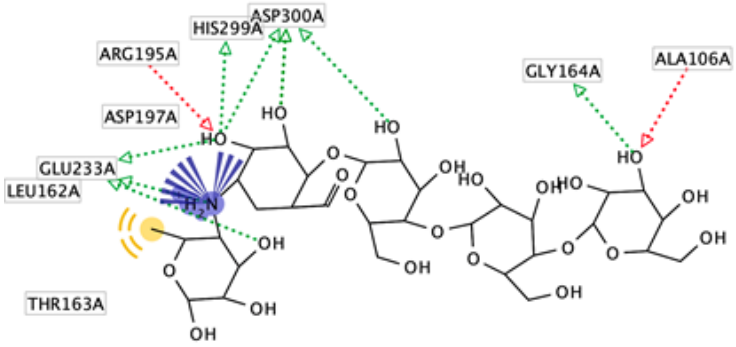

Fig. 4: The binding mode of acarbose with α-amylase. Hydrogen bond donors are shown as green dashed-lines arrows. Hydrogen bond acceptors are shown as red dashed-lines arrows. The blue highlight indicates a positive ionizable area. The yellow highlight indicates hydrophobic interaction

Aldose reductase

A 3D X-ray crystal structure of aldose reductase was employed in this work (PDB ID 1Z8A, resolution 0.95 Å in complex with a sulfonyl-pyridazine inhibitor, deposited by Steuber *et al.* [19]). The inhibitor was co-crystallized in this enzyme and interacts by building 10 hydrogen bonds (fig. 5).

Validation of the molecular docking simulation

The validation of the molecular docking simulation, carried out by re-docking the inhibitors into their original position in the active pocket of the macromolecules and superimposing the re-docked inhibitors with the original conformation of the inhibitors, resulted in RSMD values<2 Å (fig. 6).

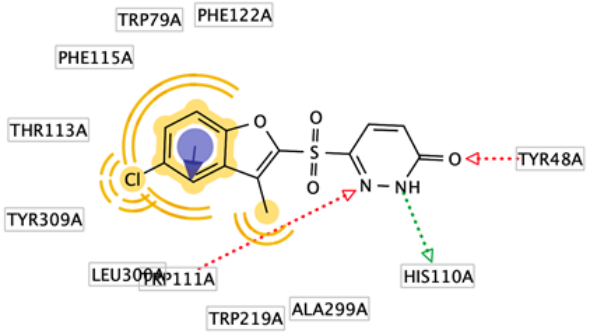

Fig. 5: The binding mode of sulfonyl-pyridazine inhibitor with aldose reductase. Hydrogen bond donors are shown as a green dashed-line arrow. Hydrogen bond acceptors are shown as red dashed-line arrows. The blue highlight indicates a positive ionizable area. The yellow highlight indicates hydrophobic interaction

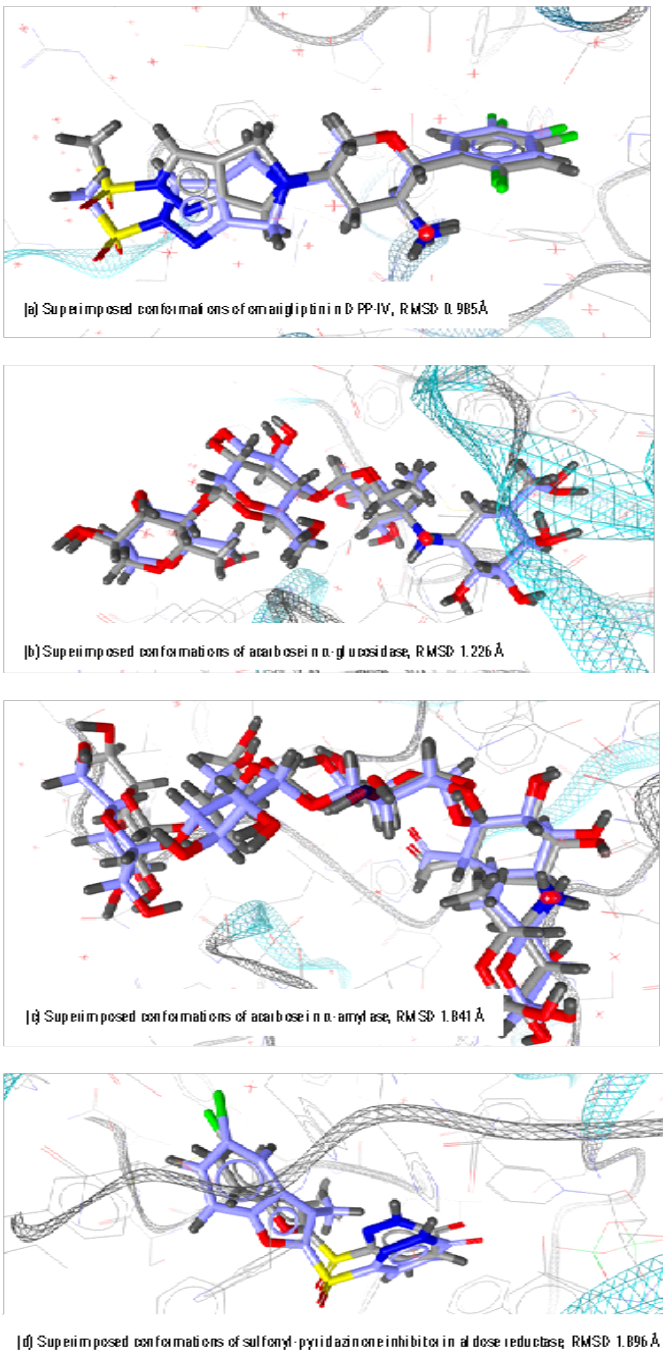

Fig. 6: Validation of the molecular docking simulation by superimposing the re-docked conformation with the original conformation of the inhibitors; (a) omarigliptins in DPP-IV; (b) acarboses in α-glucosidase; (c) acarbose in α-amylase; (d) sulfonyl-pyridazinones in aldose reductase

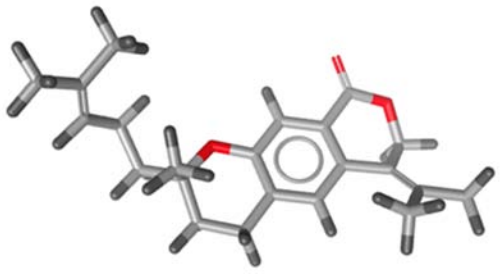

Fig. 6: The MMFF94 geometry-minimized conformation of the ligand peperochromene A, the summary of the physicochemical properties of the chromene compound and the inhibitors is presented in table 1

Physicochemical properties of the ligand

The geometry optimization using MMFF94 had reached a local minimum in the potential energy curve with a value of 64.007 kcal/mol (the geometry-minimized conformation of the ligand is depicted in fig. 6). The physicochemical properties of the ligand Peperochromene A ($C_{22}H_8O_3$, MW = 340.463) reveals 8 rotatable bonds and 6 aromatic atoms, and the hydrophobicity is represented by cLogP of 5.249.

Molecular docking simulation

Molecular docking simulation of peperochromene A isolated from *Peperomia pellucida* with four proteins involved in the homeostasis of blood glucose levels, namely DPP-IV, alpha-glucosidase, alpha-amylase, and aldose reductase, showed that peperochromen A could interact with all four targets; however, it binds to α-glucosidase and α-amylase with Ki (inhibition constant) value better than that of acarbose, the enzymes' known inhibitor. This chromene compound also reveals an inhibition constant value to aldose reductase similar to that of the enzyme inhibitor (table 2 and fig. 7).

Theorem 1. Peperochromene A isolated from the leaves of Peperomia pellucida (Piperaceae) could interact with four proteins that modulate the homeostasis of blood glucose.

Proof of Theorem 1. Peperochromene A could interact with DPP-IV (table 2 and fig. 8a), α-glucosidase (table 2 and fig. 8b), α-amylase (table 2 and fig. 8c), and aldose reductase (table 2 and fig. 8d) □

Table 1: The physicochemical properties of the peperochromene A and the inhibitors

Compound	Hydrogen bond donors	Hydrogen bond acceptors	Hydrophobicity (cLogP)
Peperochromene A ((S)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one	0	2	5.249
DPP-IV inhibitor (omarigliptin)	1	7	0.789
α-glucosidase and α-amylase inhibitor (acarbose)	14	18	-10.370
Aldose reductase inhibitor (sulfonyl-pyridazinone)	1	4	3.392

Table 2: The physicochemical properties of the ligand and the inhibitors

Compound	DPP-IV	α-glucosidase	α-amylase	Aldose reductase
	Ki (μM)	Ki (μM)	Ki (μM)	Ki (μM)
Enzyme inhibitor				
Omarigliptin	1.22	-	-	-
Acarbose	-	1172.61	6.38	-
Sulfonyl-pyridazinone	-	-	-	0.40
Peperochromene A	336.47	4.63	2.13	4.56

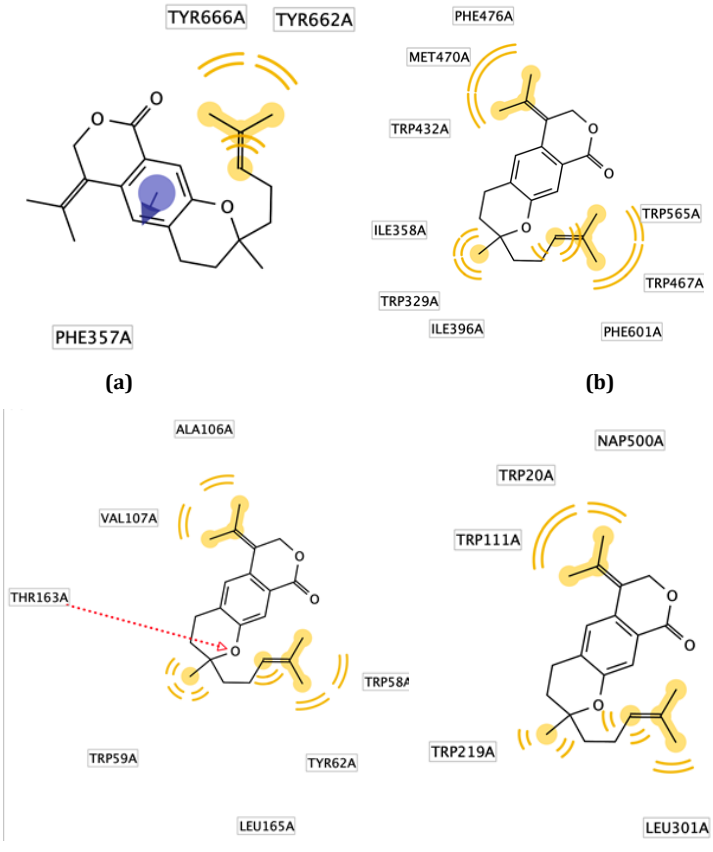

Fig. 8: The binding mode of the ligand peperochromene A with (a) DPP-IV; (b) α-glucosidase; (c) α-amylase; and (d) aldose reductase

DISCUSSION

The catalytic site of DPP-IV is situated in a small pocket centered toward the C-terminal end around Ser630 residue [19]. DPP-IV inhibitors exhibit a low risk of hypoglycemia; nonetheless, headaches, gastrointestinal distress, and skin lesions were reported in some studies [20]. DPP-IV inhibitors are recognized to interact with Glu205 and Glu206 in the S2 subsite, e. g., the amino group of sitagliptin builds 1 hydrogen bond with the hydroxyl group of Tyr662 and 3 hydrogen bonds with the carboxylate oxygens of Glu205 and Glu206 [4, 21]. Hydrophobic interaction, 2 aromatic stackings, of this inhibitor with Phe357 and Tyr662 were also reported [4]. Omarigliptin, a long-acting DPP-IV inhibitor, builds 2 hydrogen bonds with Glu205 and Tyr662 [16].

Our chromene compound, (S)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano [4,3-g]chromen-9(2H)-one or Peperochromene A, is a very hydrophobic compound as proven by its cLogP value of 5.249 with no hydrogen bond donor and only 2 hydrogen bond acceptors (fig. 1 and table 1). This chromene compound interacts with DPP-IV via 1 aromatic stacking with Phe357 and 2 hydrophobic interactions with Tyr662 and Tyr666 (fig. 8a). Regrettably, these interactions resulted in a higher inhibitory constant compared to the co-crystallized inhibitor (omarigliptin) (table 2).

α-glucosidase and α-amylase inhibitors, e. g., acarbose, reduce blood glucose by postponing the degradation and absorption of carbohydrates in the intestine [22, 23]. α-glucosidase can be weakly inhibited by flavonoids [24] anthocyanins [25], and alkaloids [26]. The active site of α-glucosidase, predicted by MOE Site Finder, was reported to consist of Gln66, Met69, Asp106, Ile109, residues 152-158, Leu174, Arg175, Arg212, Ile213, Thr215, Ala216, Pro226, residues 230-239, Trp242, Val274, Glu276, Val277, residues 300-313, Asn347, Asp349, Gln350, Asp408, Asn412, Leu437, and Arg439 [26]. Acarbose interacts with this enzyme by building 11 hydrogen bonds and 1 hydrophobic interaction with Phe601 [17].

Peperochromene A interacts with α-glucosidase by building several hydrophobic interactions, one of them being with Phe601 (fig. 8b). It interacts with α-amylase by building 1 hydrogen bond with Thr163 and 3 hydrophobic interactions with Trp58, Trp59, Tyr62, Ala106, and Val107 (fig. 8c). Peperochromene A binds to α-glucosidase and α-amylase with a Ki (inhibition constant) value better than that of acarbose (table 2). This chromene compound also binds with aldose reductase by hydrophobic interactions with Trp20, Trp111, Trp219, Leu301, and reveals a constant inhibition value similar to that of the enzyme inhibitor (table 2 and fig. 8d).

CONCLUSION

From this study it can be concluded that Peperochromene A, a novel compound isolated from *Peperomia pellucida,* is potential to be developed as an inhibitor of the proteins involved in the homeostasis of blood glucose, thus, it can be further explored for its antidiabetic activity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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