

MOLECULAR DOCKING OF β -SITOSTEROL AND STIGMASTEROL ISOLATED FROM *Morinda citrifolia* WITH α -AMYLASE, α -GLUCOSIDASE, DIPEPTIDYLPEPTIDASE-IV, AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR- γ

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ABSTRACT

In Indonesia, *Morinda citrifolia* (Rubiaceae) fruit has been traditionally used to treat various diseases. A previous study confirmed the presence of flavonoids, terpenoids, alkaloids, and steroids in the ethanol and hexane extracts of *M. citrifolia* fruit. Our work studied the molecular interaction of β -sitosterol and stigmasterol isolated from the fruit of this plant with α -amylase, α -glucosidase, PPAR- γ , and DPP-IV. The X-ray crystal structures of the proteins were downloaded from RCSB Protein Data Bank (<https://www.rcsb.org/>). The 3D structure of the ligands was obtained from the PubChem Compound databases (<https://pubchem.ncbi.nlm.nih.gov/>), subjected to energy minimization using the MMFF94 forcefield partial charges, and the torsions were set to default in the AutoDock tools program. The ligands were docked to the active binding site of the proteins. The results indicated that both stigmasterol and β -sitosterol could occupy all binding sites of the proteins, but the best interaction (indicated by docking score) is with α -amylase (better than that of acarbose) and α -glucosidase (weaker to that of acarbose). In DPP-IV, β -sitosterol occupies the S2 extensive subsite, similar to teneligliptin, a DPP-IV inhibitor. Stigmasterol builds one hydrogen bond with Val207 and occupies a similar location with β -sitosterol. β -sitosterol also interacts with Glu343 similarly as chiglitazar, a full agonist of PPAR- γ . Thus, β -sitosterol might be able to be further developed as an anti-T2DM drug candidate by inhibiting α -amylase, α -glucosidase, DPP-IV, as well as activating PPAR- γ .

Keywords: α -amylase, α -glucosidase, β -sitosterol, Diabetes mellitus, Dipeptidyl peptidase-IV, *Morinda citrifolia*, noni fruit, PPAR- γ

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INTRODUCTION

Type 2 diabetes mellitus, abbreviated as T2DM, is a chronic pancreatic disorder in which the insulin response is diminished and leads to insulin resistance. Throughout this condition, insulin is futile and is initially marked by an increase in its production to maintain glucose homeostasis, but gradually, insulin production decreases.¹ Insulin therapy is an option to control DM.² α -Amylase (an enzyme that involves the hydrolysis of glycosidic bond in starch) and α -glucosidase are two important enzymes that are targeted by their inhibitors to manage low blood glucose levels in diabetic patients.^{3,4} T2DM is also treated by regulating glucagon-like peptide-1 (GLP-1) with dipeptidyl peptidase-IV (DPP-IV) inhibitors. DPP-IV inhibitors are recognized as drugs that prolong the half-life of GLP-1, thus slow down the emptying process in the stomach and reduces appetites.⁵

The fruit of *Morinda citrifolia*, a popular plant in Asia (particularly Indonesia, Malaysia, India), has been proven to positively contain polyphenols, reducing compounds, mucilage, alkaloids, saponins, steroids, and

terpenoids.^{6,7} The water extract of *M. citrifolia* fruit mixed in a daily beverage has been proven to reduce blood glucose levels.⁷ A previous molecular docking study reported that ursolic acid isolated from the fruit of this plant showed a good affinity to α -amylase, comparable to that of acarbose.⁸ Moreover, flavonoids in *M. citrifolia* fruit, e.g. quercetin, kaempferol, nicotifloroside, narcissoside, and rutin, were reported to show good binding energy to peroxisome proliferator-activated receptor-gamma (PPAR- γ) similar to that of rosiglitazone.^{9,10}

However, there is a lack of information about the mechanism of action of β -sitosterol and stigmasterol, two phytosterols contained in *M. citrifolia* fruit. Therefore, this study aims to visualize the hydrogen bond and docking scores in the molecular interaction of β -sitosterol and stigmasterol with α -amylase, α -glucosidase, PPAR γ , and DPP-IV.

EXPERIMENTAL

Hardware and Software for Molecular Docking

The hardware used for molecular docking was a 13-inch ASUS with Intel (R) Core i5-7200 processor, 4096 MB RAM 2.50GHz, and 1TB HD, VGA NVIDIA GEFORCE 930 MX. The software used was AutoDock4 and Discovery Studio Visualizer (Freeware).¹¹

Preparation of Ligands, Proteins, and Molecular Docking

The 3D structure of β -sitosterol and stigmasterol was obtained from the PubChem Compound databases (<https://pubchemdocs.ncbi.nlm.nih.gov/compounds>), subjected to energy minimization using the MMFF94 forcefield partial charges, and the torsions were set to default in the AutoDock tools program. Geometry optimization was performed using MMFF94, which is intended to produce accurate geometric structures.¹² The x-ray crystallographic 3D structure of human pancreatic α -amylase (complexed with nitrite and acarbose (PDB ID 2QV4) crystallized and deposited by Williams et al., (2007) resolution 1.97 Å was downloaded from online Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<https://www.rcsb.org/structure/2QV4>).¹³ The protein preparation and ligand separation were carried out by following Megantara et al. procedure.^{14,15} The separated ligand was used as the standard.¹⁶ Validation was performed by following Levita et al. (2017) procedure. The ligand was re-docked into its original location, followed by superimposing the re-docked ligand with the co-crystallized ligand that had been separated from the protein.¹⁷

The x-ray crystallographic 3D structures of human lysosomal acid- α -glucosidase (GAA) in complex with acarbose (PDB ID 5NN8) crystallized and deposited by Roig-Zamboni et al., (2017) resolution 2.45 Å was retrieved from online Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<https://www.rcsb.org/structure/5NN8>).¹⁸ The x-ray crystallographic 3D structures of human DPP-IV/CD26 complexed with its inhibitor, omarigliptin, (PDB ID 4PNZ) crystallized and deposited by Scapin and Yan (2014) resolution 1.90 Å was retrieved from online Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<https://www.rcsb.org/structure/4PNZ>).¹⁹ The x-ray crystallographic 3D structures of human PPAR- γ -ligand-binding domain complexed with an indole-based modulator (PDB ID 2P4Y) crystallized and deposited by McKeever (2007) resolution 2.25 Å was retrieved from online Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<https://www.rcsb.org/structure/2P4Y>).²⁰

All proteins and ligand separation were prepared by following Megantara et al. (2021) procedure.^{14,15} The separated ligand was used as the standard.¹⁶ Molecular docking was carried out for β -sitosterol and stigmasterol towards the binding site of the proteins by employing AutoDock. The flexibility of the proteins was regulated by setting the scaling factor for the nonpolar atoms to 0.8. All other parameters were fixed at the default condition. The binding affinity of the protein/ligand complexes was expressed in terms of docking scores.

RESULTS AND DISCUSSION

Initially, the molecular docking method was validated by superimposing the co-crystallized ligands with the extracted ligands from the complexed structures and re-docked them into their original location in the protein structure (depicted in Fig.-1). The molecular docking procedure was confirmed valid and reliable with root mean square deviation, RMSD, < 2.00 Å. The re-docked poses are nearly overlapped with the co-crystallized conformations (Fig.-1).

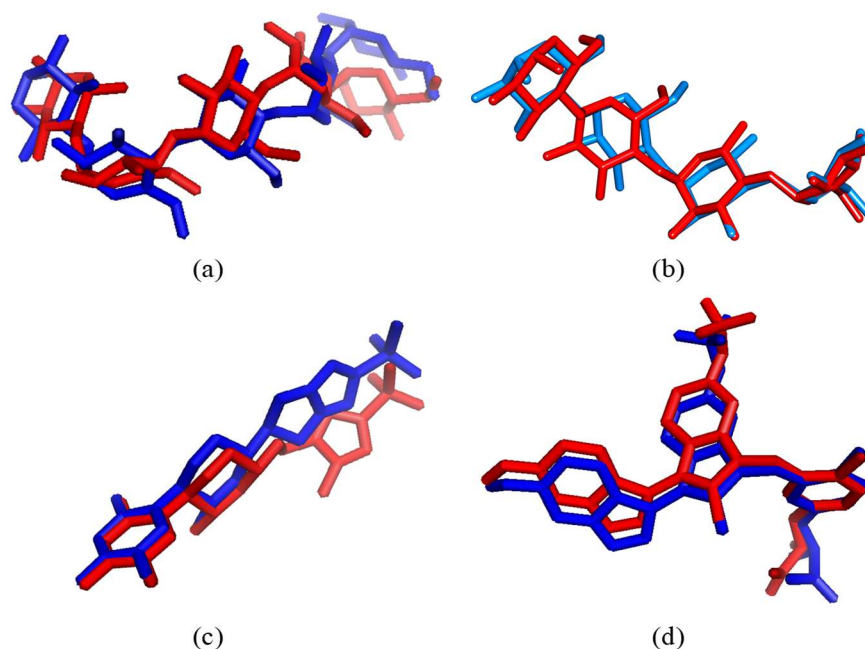


Fig.-1: Superimposed Structures of the Re-docked Ligand with the Co-crystallized Ligand Separated from the Protein: (a) Acarbose in Human Pancreatic α -Amylase (RMSD = 0.571 Å); (b) Acarbose in Human Lysosomal Acid- α -Glucosidase (RMSD = 1.80 Å); (c) Omarigliptin in DPP-IV (RMSD = 1.838 Å); (d) Indole-based Modulator in PPAR- γ (RMSD = 1.046 Å)

The re-docking of acarbose into its original location in human pancreatic α -amylase reveals that this compound interacts by building 16 hydrogen bonds with important residues Trp59, Tyr62, Gln63, His101, Asn105, Ala106, Val107, Thr163, Gly164, Arg195, Glu233, His299, Asp300 (Fig.-2).

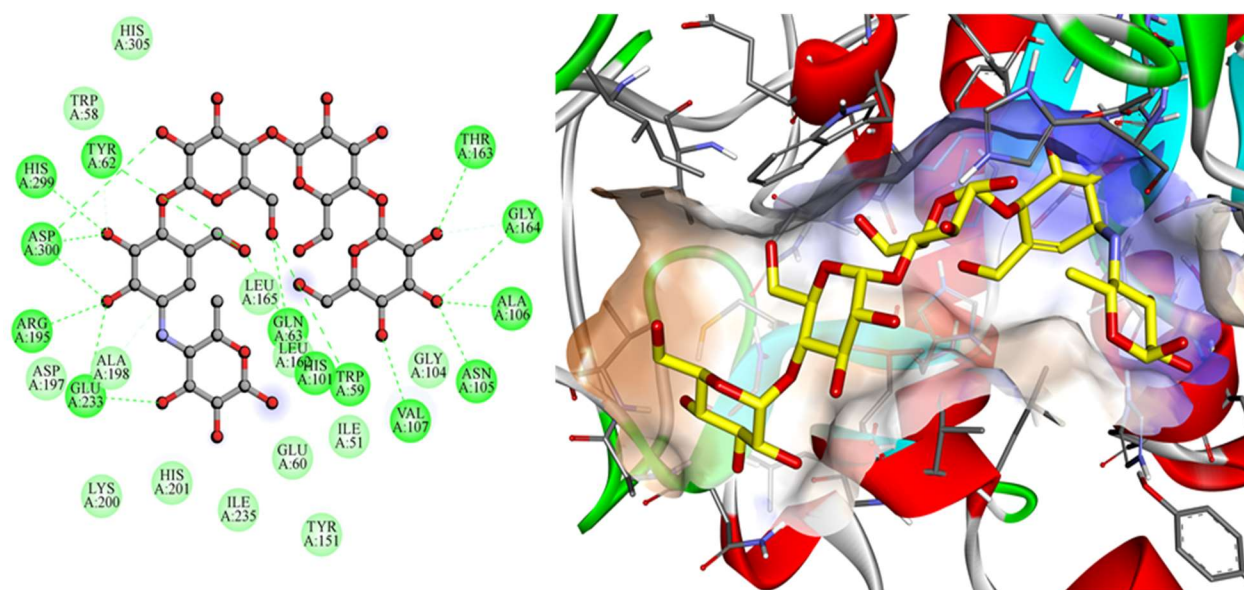


Fig.-2: Re-docking of Acarbose (yellow and red structure) into Its Original Location in Human Pancreatic α -Amylase. Acarbose Builds 16 Conventional Hydrogen Bonds with Amino Acid Residues (depicted as circles in the left side picture). The Green Circles Illustrate the Conventional Hydrogen Bonds with Polar Amino Acid Residue, the Van der Waals Interaction with Non-polar Amino Acid Residue is the Pale Turquoise Circles.

It was reported that α -amylase, in general, possesses a carbohydrate-binding module and a surface-binding site, both non-catalytic, to facilitate the binding of its substrate.²¹

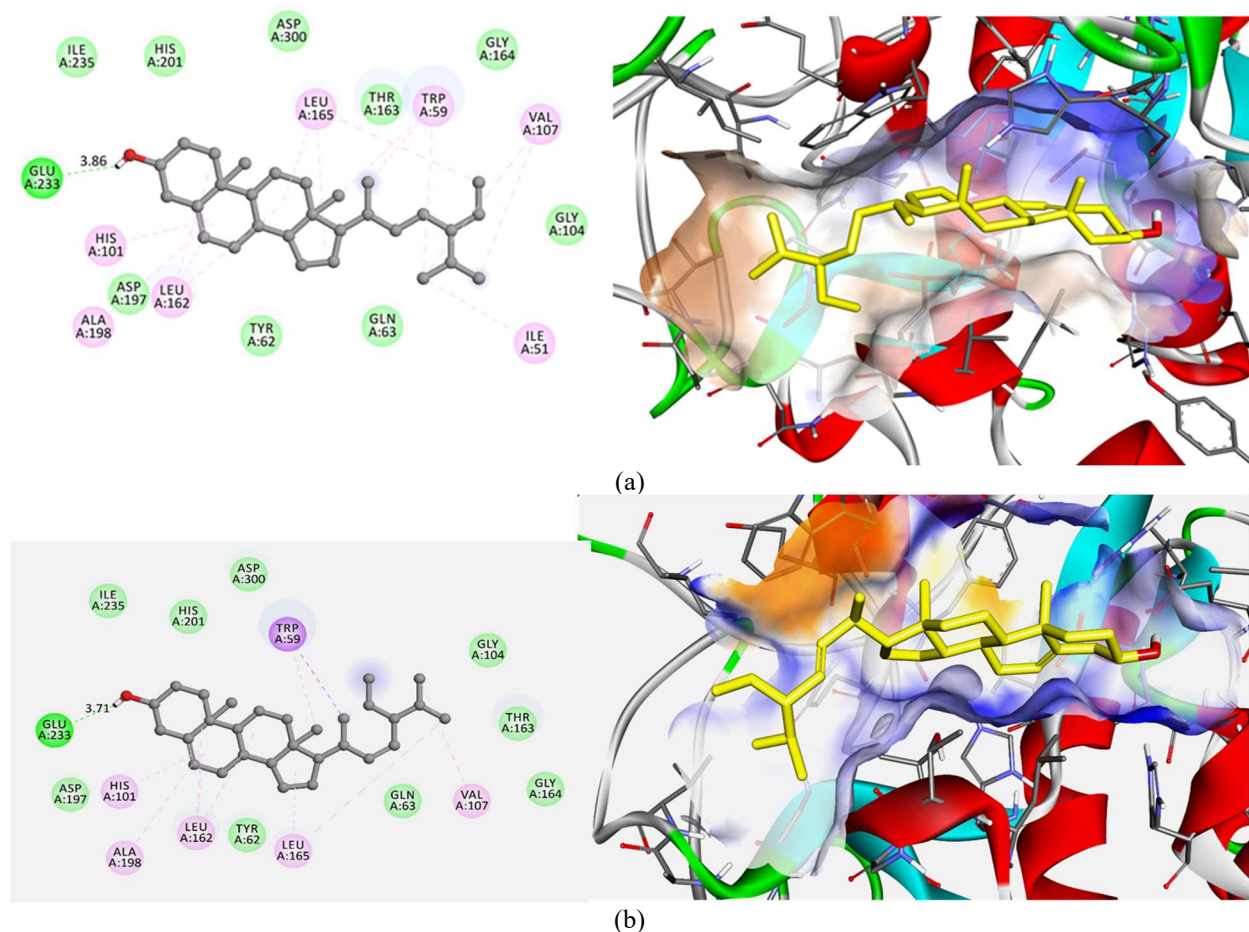


Fig.-3: Molecular Docking of (a) β -sitosterol and (b) Stigmasterol into the Binding Site of Human Pancreatic α -Amylase. Only One Conventional Hydrogen Bond is Detected Between Both Phytosterols and Glu233. Stigmasterol Builds Hydrophobic Interaction with Trp59.

The docking of β -sitosterol and stigmasterol into the binding site of human pancreatic α -amylase is presented in Fig.-3 and Table-1. Although both β -sitosterol and stigmasterol only interact with Glu233 by building one hydrogen bond, these compounds show a better binding affinity (docking score = -8.82 kcal/mol for β -sitosterol and -8.86 kcal/mol for stigmasterol) with the enzyme compared to that of acarbose (docking score = -8.59 kcal/mol). Moreover, stigmasterol builds hydrophobic interaction with Trp59. The re-docking of acarbose into its original location in human lysosomal acid- α -glucosidase reveals that this compound interacts by building hydrogen bonds with important residues Asp282, Asp404, Arg600, Asp616, His674 (Fig.-4).

The docking of β -sitosterol and stigmasterol into the binding site of human lysosomal acid- α -glucosidase is presented in Fig.-5 and Table-1. β -sitosterol only interacts with Asn524 by building one hydrogen bond and shows a weaker binding affinity (docking score = -8.24 kcal/mol) with the enzyme compared to that of acarbose (docking score = -8.47 kcal/mol). However, stigmasterol does not build any hydrogen bond with amino acid residues in the binding site of human lysosomal acid- α -glucosidase.

Our molecular docking result is relevant to a previous study by Ponnulakshmi and co-workers. It was reported that β -sitosterol could normalize the blood glucose level of diabetes-induced rats.²² Moreover, twenty-one days of treatment with various doses of β -sitosterol could lessen the level of glucose in diabetic rats.²³

The re-docking of omarigliptin into its original location in DPP-IV reveals that this compound interacts by building hydrogen bonds with important residues Arg125, Glu205, Glu206, Tyr547, Tyr662 and 2 aromatic stackings with Phe357 and Tyr366 (Fig.-6).

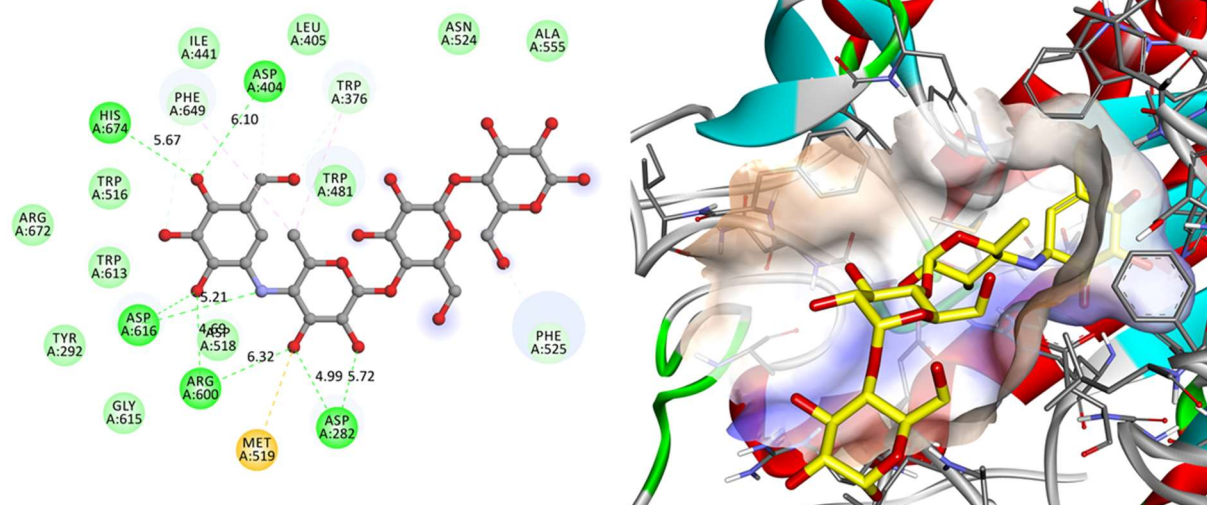


Fig.-4: Re-docking of Acarbose (yellow and red structure) into Its Original Location in Human Lysosomal Acid- α -Glucosidase. Acarbose Builds 9 Conventional Hydrogen Bonds with Amino Acid Residues (depicted as circles in the left side picture).

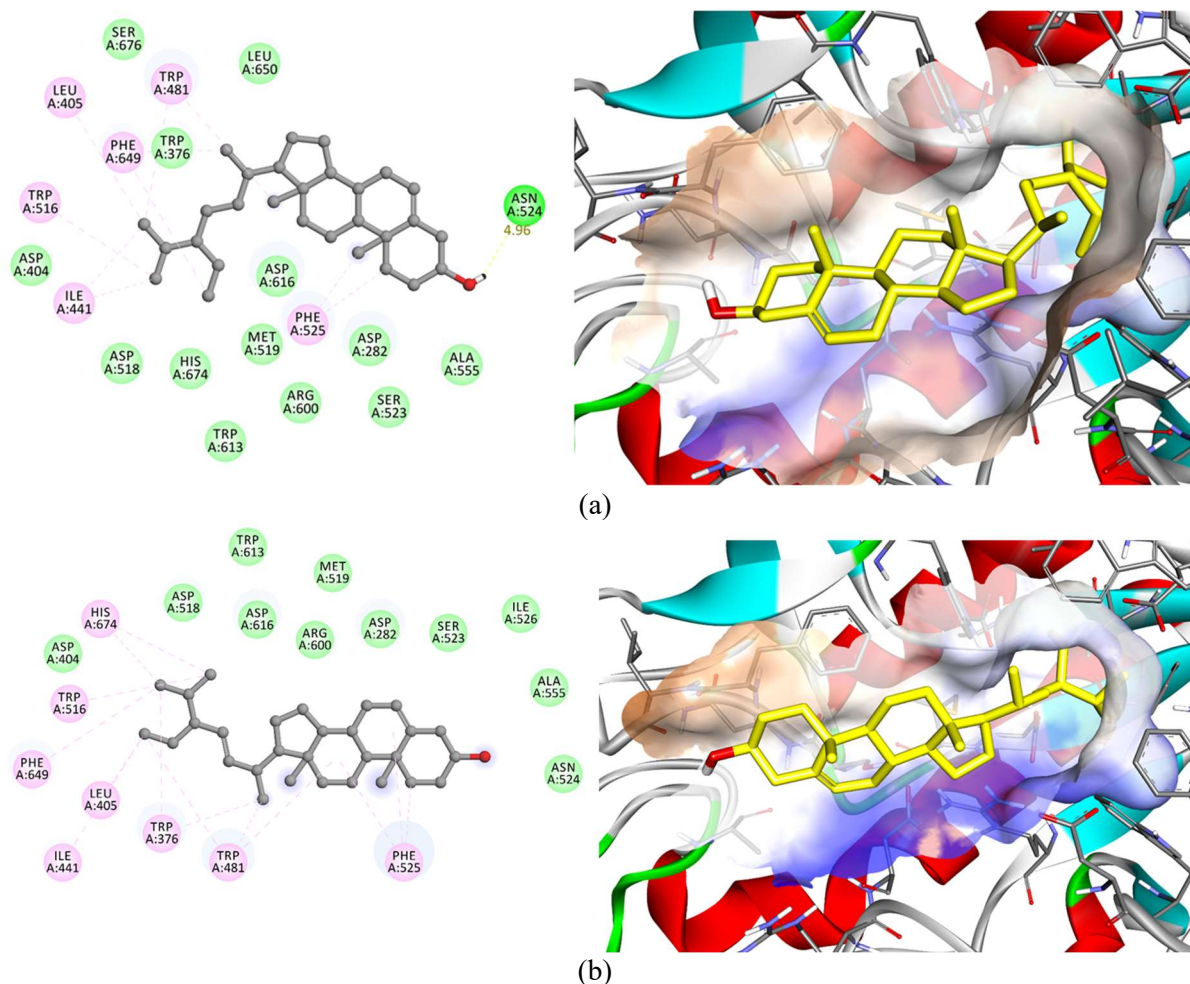


Fig.-5: Molecular Docking of (a) β -sitosterol and (b) Stigmasterol into the Binding Site of Human Lysosomal Acid- α -Glucosidase. Only One Conventional Hydrogen Bond is Detected between β -sitosterol and Asn524. No Hydrogen Bond is built between Stigmasterol and Amino Acid Residues in the Binding Site of Human Lysosomal Acid- α -Glucosidase.

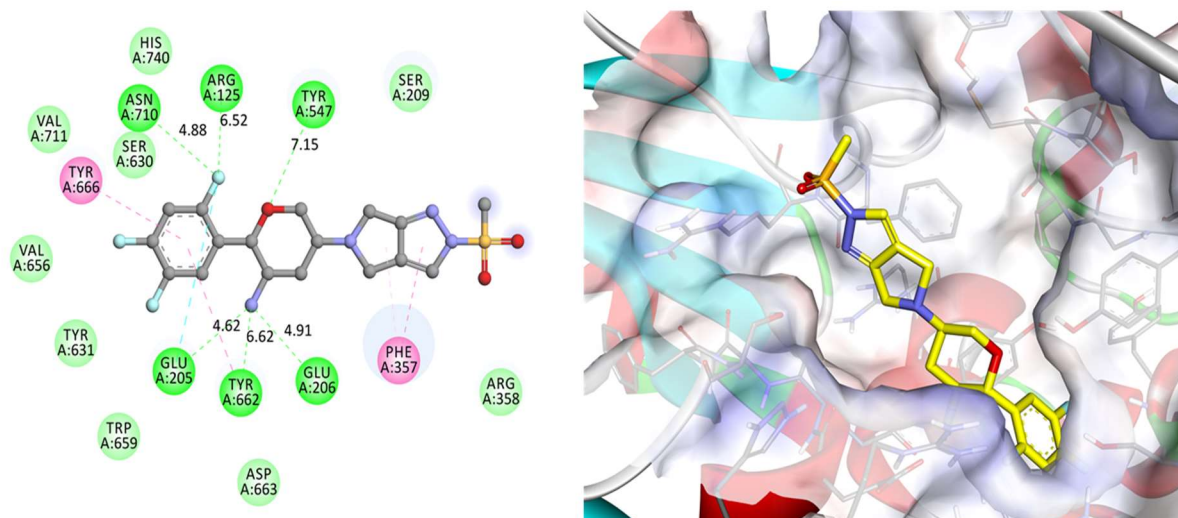
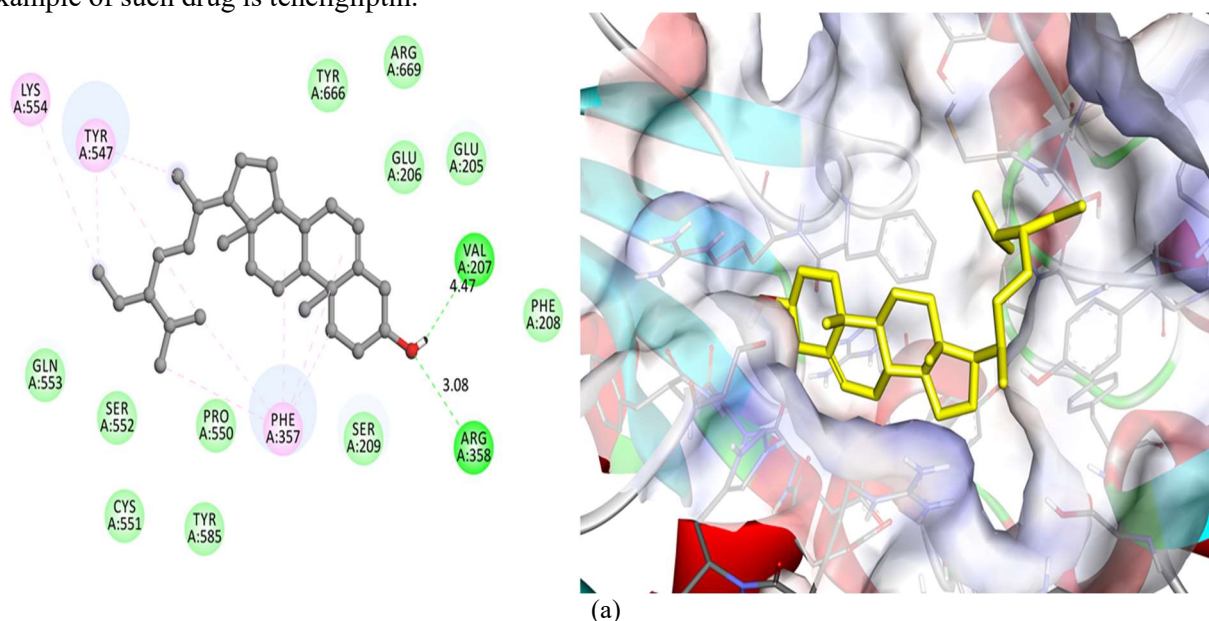


Fig.-6: Re-docking of Omarigliptin (yellow and red structure) into Its Original Location in Human DPP-IV. Omarigliptin Builds 8 Conventional Hydrogen Bonds and 2 Aromatic Stackings with Amino Acid Residues (depicted as circles in the left side picture).

The molecular interaction of omarigliptin is compared to a previous study by Aulifa and colleagues (2019), about *in silico* study of sitagliptin, a known inhibitor of DPP-IV.⁵ Related to our result, Aulifa reported that sitagliptin also interacts with Glu205, Glu206, Tyr662. Sitagliptin built two aromatic stackings with Phe357 and Tyr662,⁵ whereas omarigliptin in our study also forms two aromatic stackings with Phe357 and Tyr662. The active binding site of DPP-IV comprises peptide subsites, which are occupied by the respective amino acids and are numbered as S2 (assembled by Arg125, Phe357, Arg358, Glu205, Glu206, Arg669), S1 (assembled by Ser630, Val656, Trp659, Tyr662, Tyr666, Val711, Asn710), S'1 (assembled by Phe357, Tyr547, Pro550, Ser630, Tyr631, Tyr666), and S'2 (assembled by Tyr547, Trp629, Ser630, His740). The N-terminus of the substrate peptide is perceived by Glu205, Glu206, and Ser630. On top of these subsites, another site across S2, namely the S2-extensive subsite, is predicted to imply a role in the interaction. This site is composed of Val207, Ser209, Phe357, and Arg358.²⁴

Unlike to the gliptin drugs that interact with DPP-IV in the S2-S1 subsites (e. g. sitagliptin, linagliptin, alogliptin, and 4-hydroxyderricin),^{5,24} a drug can inhibit DPP-IV by occupying the S2-extensive subsite, the example of such drug is teneligliptin.²



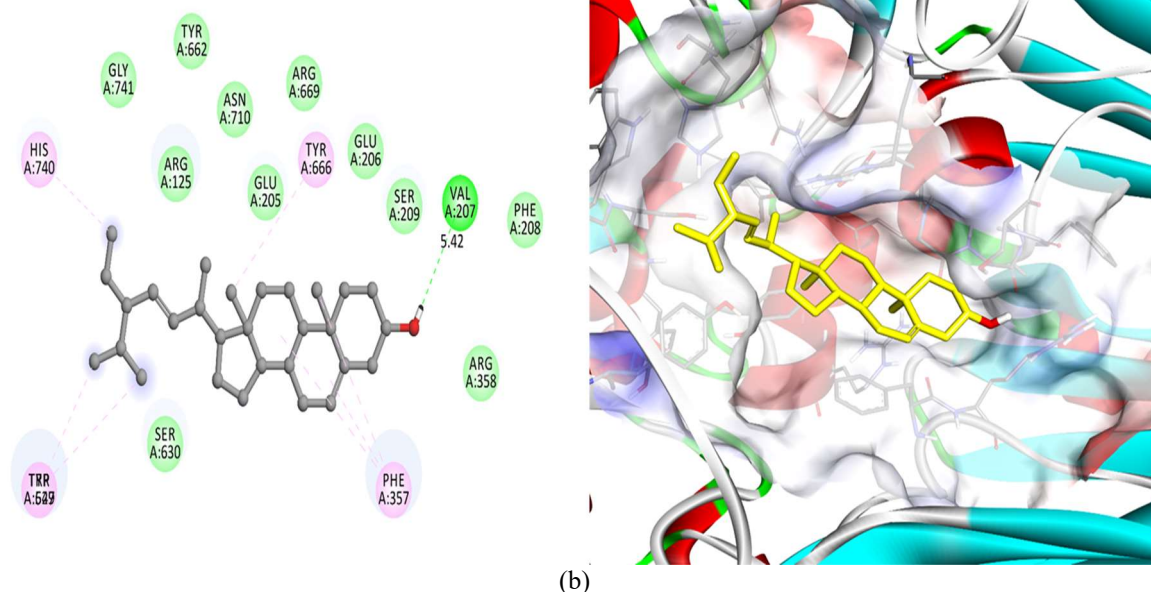


Fig.-7: Molecular Docking of (a) β -sitosterol and (b) Stigmasterol into the Binding Site of Human DPP-IV. Two Conventional Hydrogen Bonds are Detected between β -sitosterol and Val207 and Arg358, while Only One Hydrogen Bond is Built between Stigmasterol and Val207.

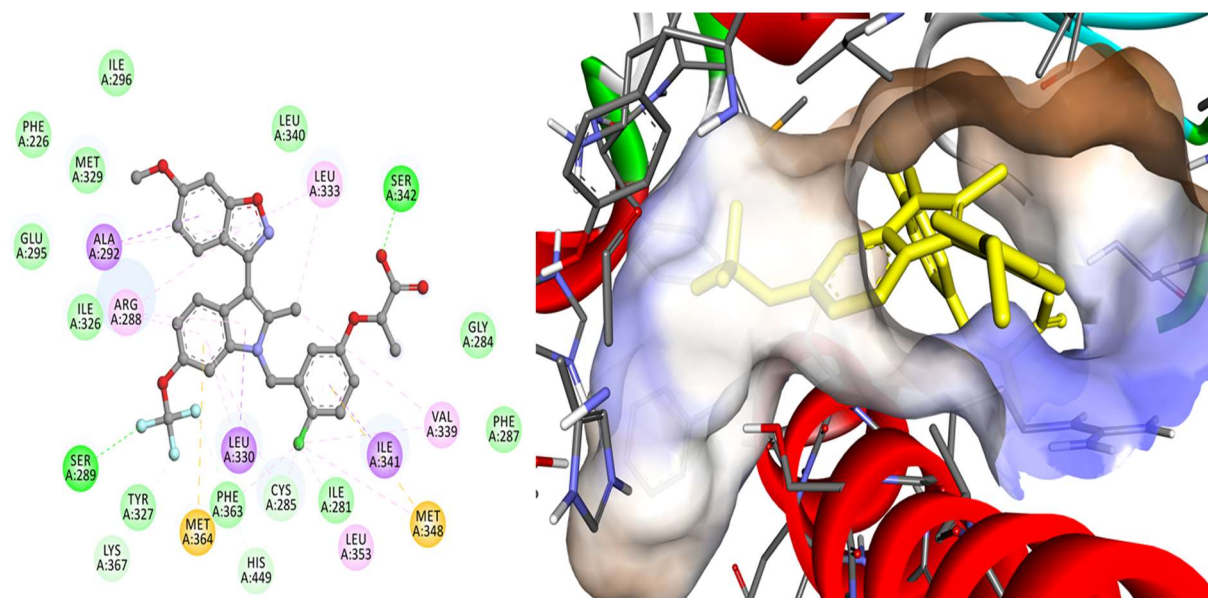


Fig.-8: Re-docking of an Indole-based Modulator (yellow and red structure) into Its Original Location in Human PPAR- γ . The Modulator Builds 2 Conventional Hydrogen Bonds and 3 Aromatic Stacking with Amino Acid Residues (depicted as circles in the left side picture).

The docking of β -sitosterol and stigmasterol into the binding site of human DPP-IV is presented in Fig.-7 and Table-1. β -sitosterol only interacts with Val207 and Arg358, which implies that this compound occupies the S2 extensive subsite of DPP-IV, matching that of teneligliptin. However, β -sitosterol shows a weaker binding affinity (docking score = -9.95 kcal/mol) with the enzyme compared to that of the standard, omarigliptin (docking score = -6.70 kcal/mol). Stigmasterol builds one hydrogen bond with Val207 and occupies a similar location with β -sitosterol, that is in the S2 extensive subsite of DPP-IV.

The re-docking of an indole-based modulator into its original location in human PPAR- γ reveals that this compound interacts by building hydrogen bonds with important residues Ser289 and Ser342 and 3 aromatic stackings with Ala292, Leu330, and Ile341 (Fig.-8).

The docking of β -sitosterol and stigmasterol into the binding site of human PPAR- γ is presented in Fig.-9 and Table-1.

Figure-9 visualizes that β -sitosterol only builds unfavorable interaction with Glu343 and shows a weaker binding affinity (docking score = -9.74 kcal/mol) with the protein compared to that of the modulator (docking score = -12.40 kcal/mol). No interaction is observed for stigmasterol.

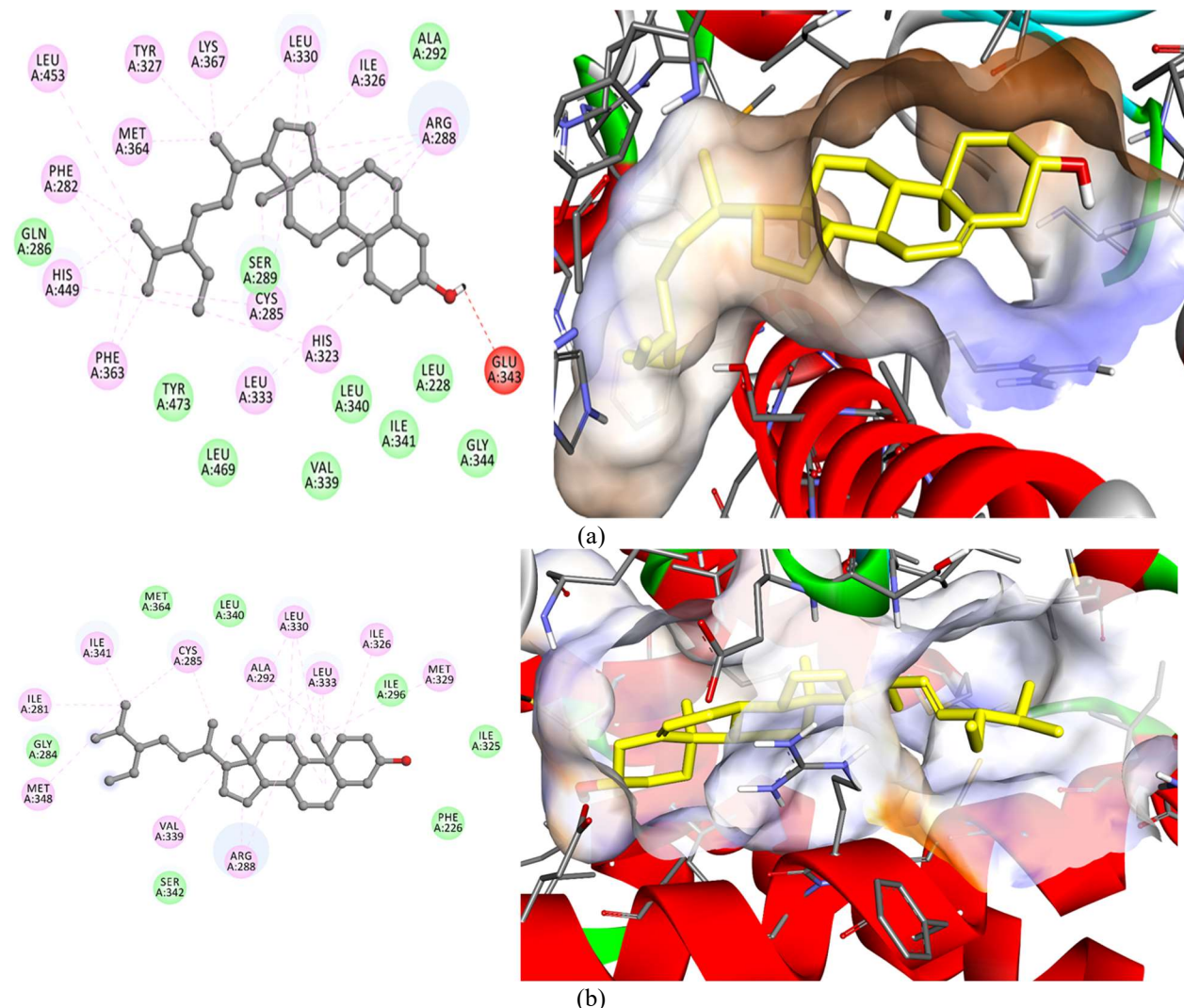


Fig.-9: Molecular Docking of (a) β -sitosterol and (b) Stigmasterol into the binding site of Human PPAR- γ . No Conventional Hydrogen Bond is Detected between either β -sitosterol or Stigmasterol and the Protein. One Unfavorable Interaction between β -sitosterol and Glu343 is detected (indicated by a red circle).

Table-1: The binding mode and affinity of β -sitosterol with the proteins compared to the natural ligand

Compound	Parameter	Binding Affinity in terms of Docking Score (kcal/mol) and Amino Acid Residues Involved in the Interaction			
	Name of the protein	Human pancreatic α -amylase	Human lysosomal acid- α -glucosidase	Human DPP-IV	Human PPAR- γ
β -sitosterol	Docking score (kcal/mol)	-8.82	-8.24	-6.70	-9.74
	Amino acid residue involved in hydrogen bond interaction	Glu233*	Asn524	Val207, Arg358	No hydrogen bond is detected

				(located in the S2 extensive subsite)	but there is one unfavorable interaction with Glu343
Stigmasterol	Docking score (kcal/mol)	-8.86	-7.68	-7.58	-10.04
	Amino acid residue involved in hydrogen bond interaction	Glu233*	No hydrogen bond is detected	Val207	No hydrogen bond is detected
Acarbose (inhibitor of pancreatic α -amylase and lysosomal acid- α -glucosidase)	Docking score (kcal/mol)	-8.59	-8.47		
	Amino acid residue involved in hydrogen bond interaction	Trp59, Tyr62, Gln63, His101, Asn105, Ala106, Val107, Thr163, Gly164, Arg195, Glu233*, His299, Asp300	Asp282, Asp404, Arg600, Asp616, His674		
Omarigliptin (inhibitor of DPP-IV)	Docking score (kcal/mol)			-9.95	
	Amino acid residue involved in hydrogen bond interaction			Arg125, Glu205, Glu206, Tyr547, Tyr662 (located in the S1-S2 subsites)	
Indole-based modulator	Docking score (kcal/mol)				-12.40
	Amino acid residue involved in hydrogen bond interaction				Ser289, Ser342 and 3 aromatic stackings with Ala292, Leu330, Ile341

Our result is compared to a previous study by Ranjith and Vismanath (2019) about β -sitosterol isolated from *Ipomoea mauritiana*. In their work, this sitosterol had shown an excellent binding affinity to PPAR- γ with a docking score of -12.55 kcal/mol using ArgusDock software.²⁵ An *in silico* study on chiglitazar, a promising new-generation insulin sensitizer and full activation of PPAR- γ , revealed that this drug interacts with Glu343 in human PPAR- γ for 83% of the simulation period.²⁶ This similar interaction with chiglitazar, particularly the binding to Glu343, describes that our β -sitosterol might also be potential as an agonist of PPAR- γ , thus be able to activate PPAR- γ .

Moreover, a previous study reported that thirty days treatment of β -sitosterol had been confirmed in enhancing PPAR- γ gene expression in diabetic rats, which is comparable to that of metformin.²⁷ However, stigmasterol was reported for its lack of antidiabetic activity compared to that of β -sitosterol. The biological activity of stigmasterol is rather to its anti-inflammatory effect than its antidiabetic effect.²⁸

CONCLUSION

Both β -sitosterol and stigmasterol could occupy all binding sites of the proteins, but the best interaction (indicated by docking score) is with α -amylase (better than that of acarbose) and α -glucosidase (similar to

that of acarbose for β -sitosterol, weaker than acarbose for stigmasterol). In DPP-IV, both β -sitosterol and stigmasterol occupy the S2 extensive subsite, similar to teneligliptin, a DPP-IV inhibitor. β -sitosterol also interacts with Glu343, matching that of chiglitazar, a full agonist of PPAR- γ . No interaction is observed between stigmasterol and PPAR- γ . Of these two phytosterols, β -sitosterol might be able to be further developed as an anti-T2DM drug candidate which works by inhibiting α -amylase, α -glucosidase, DPP-IV, as well as activating PPAR- γ .

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