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Exploring the biomolecular mechanisms of ashitaba (*Angelica keiskei*) compounds against type 2 diabetes mellitus identified using network pharmacology and molecular docking

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

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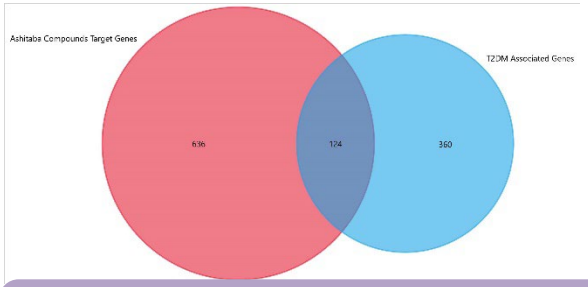
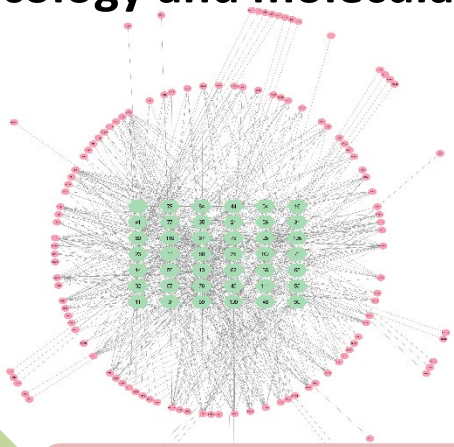
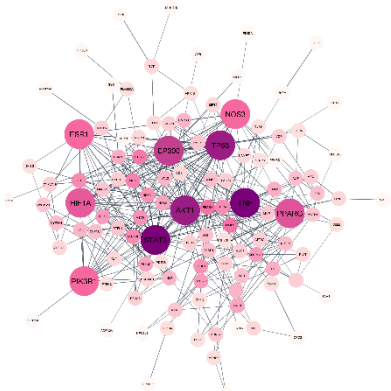
Biomolecular mechanisms of the reported Ashitaba (*Angelica keiskei*) compounds against type 2 diabetes mellitus identified using network pharmacology and molecular docking

Graphical Abstract



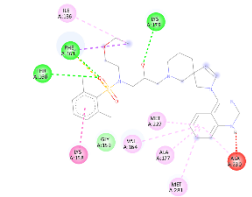
AK compounds review and target identification





Network construction (compound target and protein-protein interaction)

Gene Ontology and KEGG Pathways Enrichment Analysis



Molecular Docking

Targets of Ashitaba Compounds:
TNF-α, STAT3, p53, AKT1, HAT p300, PPAR-γ and COX-2

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and/or a dysfunctional β-cells. In the Philippines, 3.7 million people were reported to have the disease in 2017 and at least 100 deaths are caused by diabetes-related complications daily. The goal of T2DM management is to maintain blood glucose at normal physiologic levels. While drugs like oral hypoglycemic agents and insulin are available, the use of herbal medicine by T2DM patients to treat the disease is prevalent. One of the most common herbal medicines used to alleviate the symptoms of T2DM is ashitaba (*Angelica keiskei*). In the Philippines, ashitaba is commonly marketed as a tea and over 50 ashitaba-containing preparations are registered under the Philippine FDA. Since T2DM is a chronic disorder, consumption of such herbal preparation can lead to possible drug-herb interaction that may alter the pharmacokinetics and pharmacodynamics of drugs used in the management of T2DM. Hence, insights on the biomolecular mechanisms of ashitaba against T2DM is essential to determine possible drug-herb interactions if any, or to rationalize its therapeutic use. Through network pharmacology and molecular docking, reported ashitaba compounds are found to target TNF-α, STAT3, p53, AKT1, HAT p300, PPAR-γ and COX-2 in T2DM. Because of these biomolecular mechanisms, consumption of ashitaba compounds can possibly synergize or antagonize the effects of drugs used in the management of T2DM if taken concomitantly.

Keywords: ashitaba, *Angelica keiskei,* diabetes, T2DM, network pharmacology

Introduction

T2DM

- Chronic metabolic disorder
- Resistance to insulin, dysnfunctional β-cells
- Hyperglycemia → neuropathy, nephropathy, retinopathy
- 9th leading cause of death worldwide (1.5 million deaths)
- 3.7 million FILIPINO diabetics (2017) and at least 100 deaths
- GOAL: control blood sugar levels and prevent complications

Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci*. 2020;21(17):6275. doi:10.3390/ijms21176275

Regional Committee for Europe 71st session. *Seventy-First Regional Committee for Europe: Virtual Session, 13–15 September 2021: Reducing the Burden of Noncommunicable Diseases through Strengthening Prevention and Control of Diabetes*. World Health Organization. Regional Office for Europe; 2021. Accessed July 22, 2022. https://apps.who.int/iris/handle/10665/344627

Ng JYS, Clement IJ, Jimeno C, et al. Estimating direct medical costs of type 2 diabetes mellitus in the Philippines: a protocol. *BMJ Open*. 2020;10(7):e025696. doi:10.1136/bmjopen-2018-025696

World Health Organization. Diagnosis and management of type 2 diabetes (HEARTS-D). Published online 2020.

Introduction

Ashitaba (*Angelica keiskei)* (abbreviated as AK)

- Antioxidant, anti-inflammatory, antihypertensive, antidiabetic
- Over 50 products currently registered under the Philippine FDA containing AK
- Chalcones (4-hydroxyderricin and xanthoangelol)
  - Inhibition of tyrosine phosphatase 1B (PTP1B), alpha-glucosidase inhibition, activation of GLUT-4 in skeletal muscle cells and upregulation of adiponectin mRNA.
- Much of the compounds are yet to be studied and their bioactivities at the molecular level warrants elucidation
- Possible drug-herb interaction because of the chronic nature of T2DM

Caesar LK, Cech NB. A Review of the Medicinal Uses and Pharmacology of Ashitaba. *Planta Med*. 2016;82(14):1236-1245. doi:10.1055/s-0042-110496

Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetol Metab Syndr*. 2017;9:59. doi:10.1186/s13098-017-0254-9

Introduction

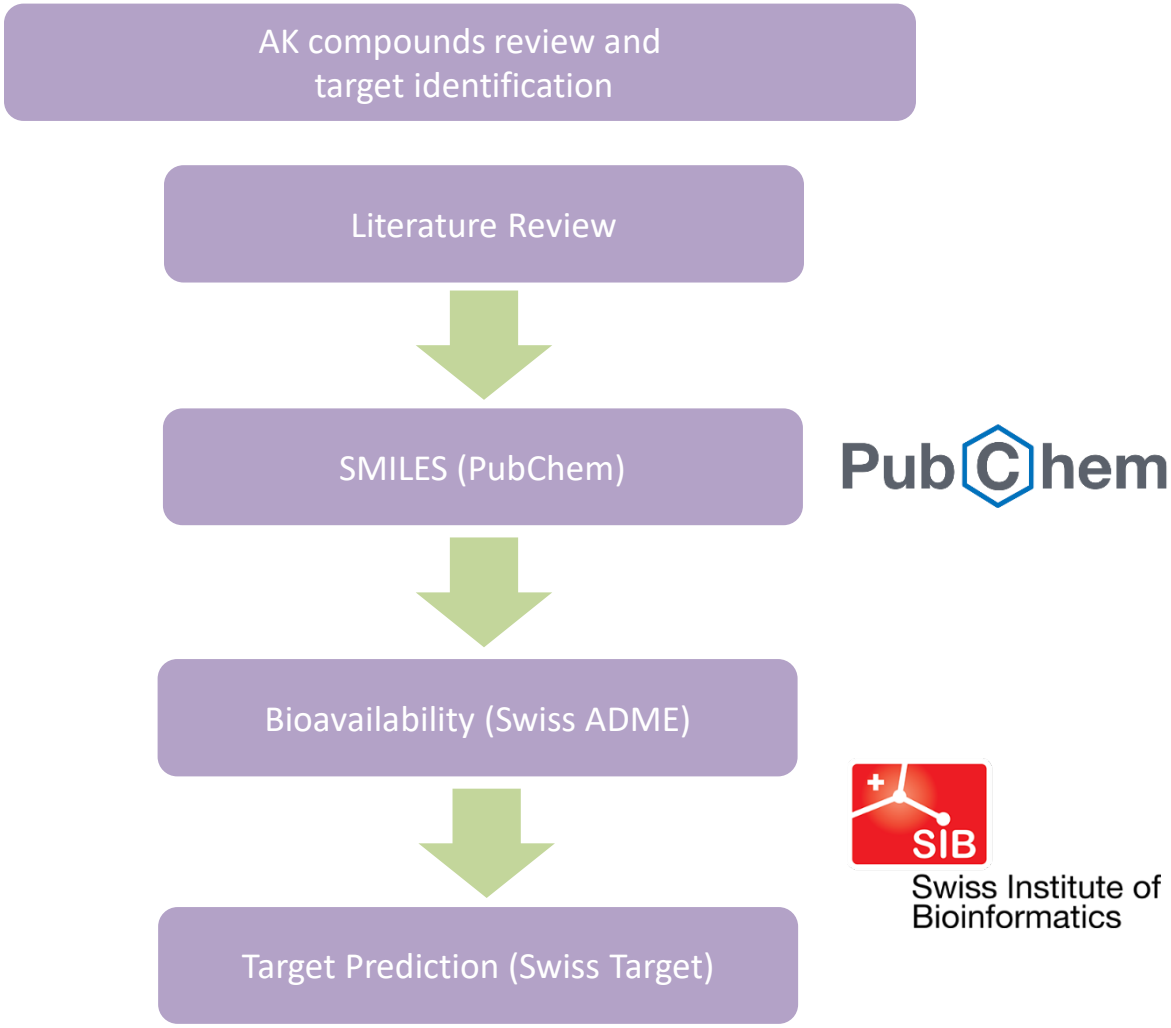
Network Pharmacology

- Integration of biology and polypharmacology via computational methods
- Compound-target-pathway networks analysis

Chandran U, Mehendale N, Patil S, Chaguturu R, Patwardhan B. Network Pharmacology. *Innovative Approaches in Drug Discovery*. Published online 2017:127-164. doi:10.1016/B978-0-12-801814-9.00005-2

Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HHHW. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*. 2022;43(2):136-150. doi:10.1016/j.tips.2021.11.004

Methods



L. Zhang *et al.*, "Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking," *Computational Biology and Chemistry*, vol. 94, p. 107396, Oct. 2021, doi: 10.1016/j.compbiolchem.2020.107396.

Methods

T2DM Genes (MalaCards)

Venn Diagram with AK Targets

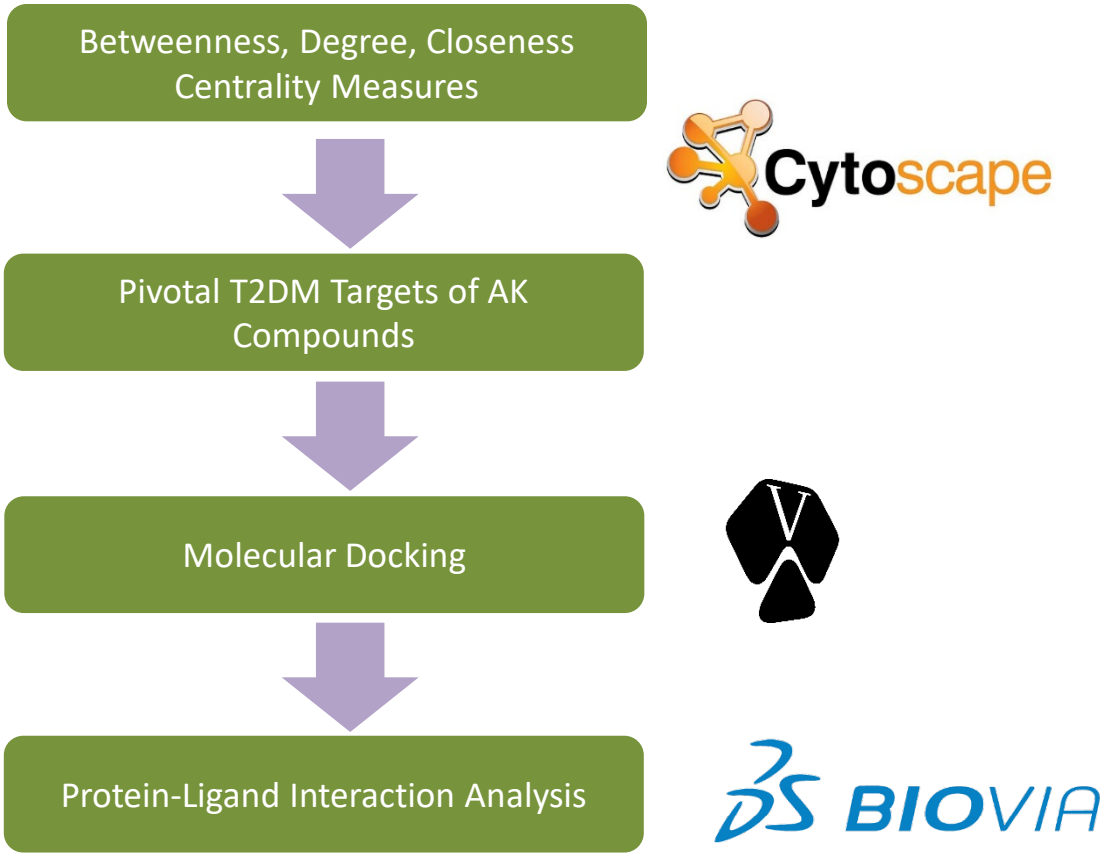
Compound-Target Network Construction

Protein-Protein Interaction Analysis

Gene Ontology and KEGG Pathway Enrichment Analysis

L. Zhang *et al.*, "Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking," *Computational Biology and Chemistry*, vol. 94, p. 107396, Oct. 2021, doi: 10.1016/j.compbiolchem.2020.107396.

Methods



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Results and discussion

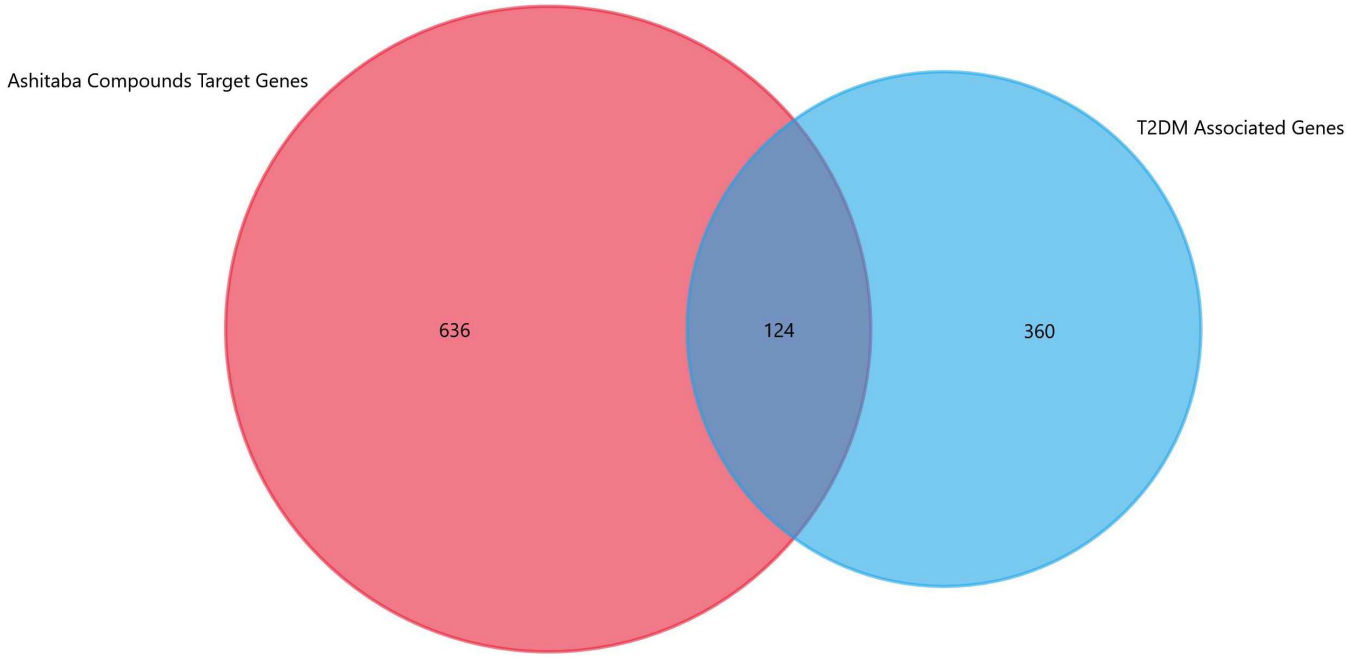

Figure 1. Venn diagram of genes targeted by the reported ashitaba compounds, and the genes associated with type 2 diabetes mellitus.

Results and discussion

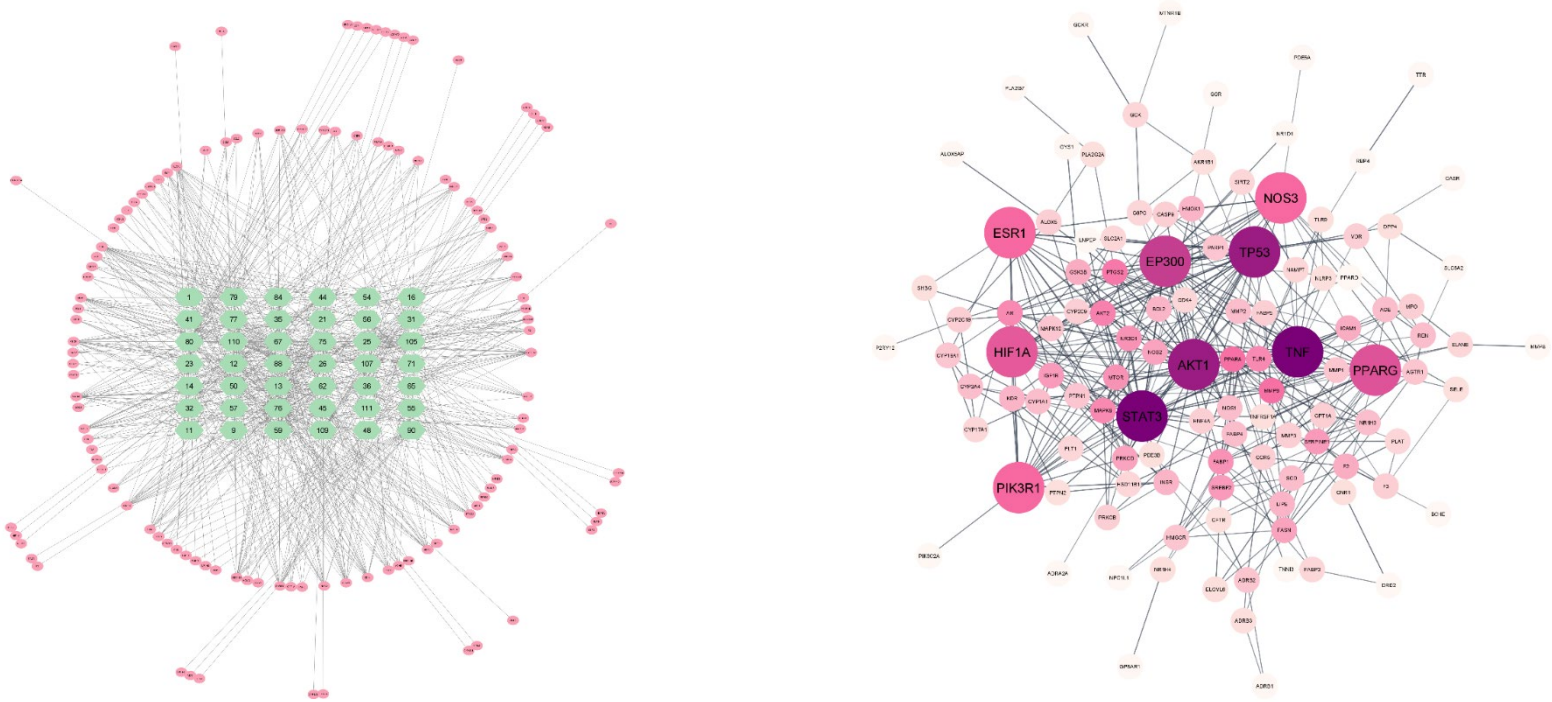

Figure 2. Compound-target network (left): the pink colored nodes represent the targets whereas the green colored nodes represent each AK compound whereas the lines represent the nondirectional interactions. The protein-protein interaction network (right): the darker the color and the bigger the node, the higher are the centrality measures (betweenness, degree and closeness).

Results and discussion

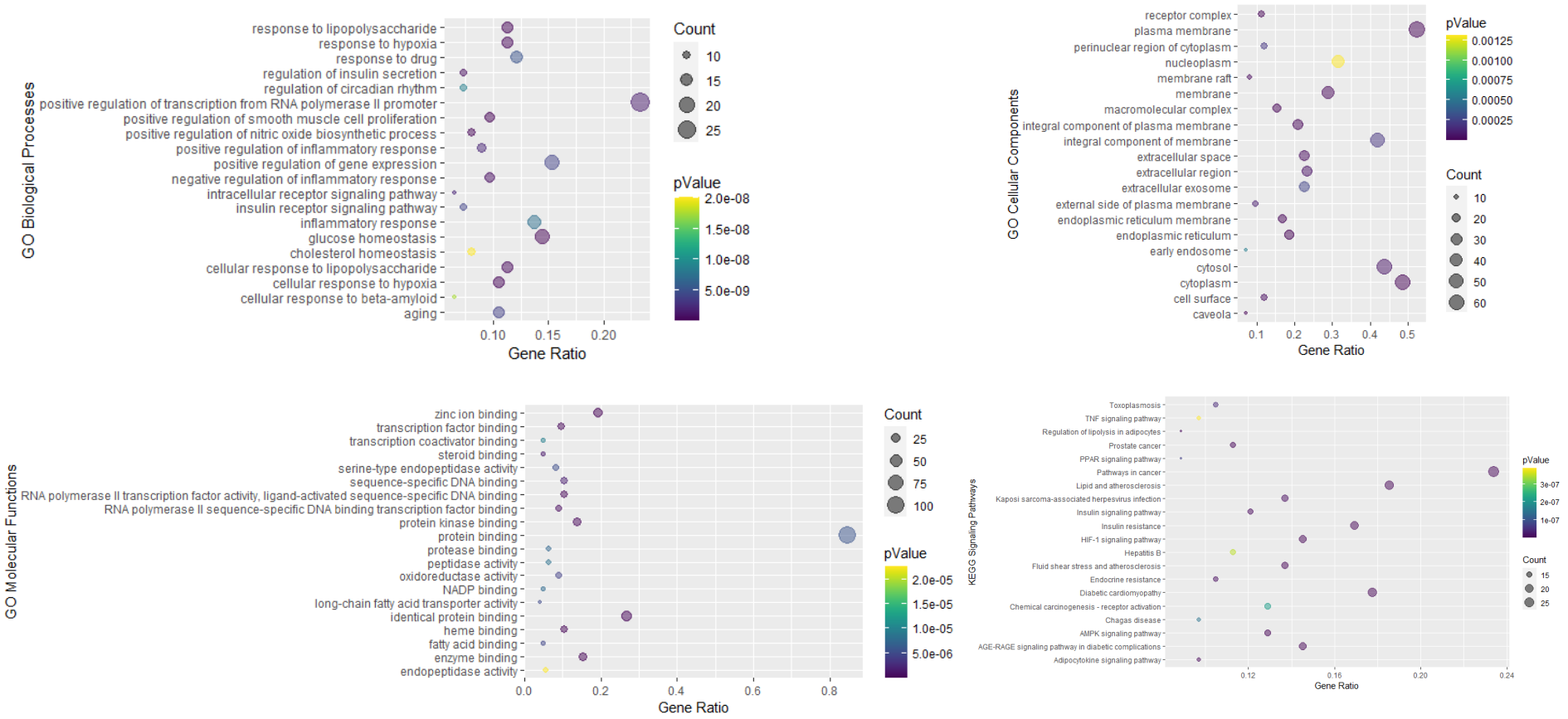

Figure 3. Bubble plot of gene ontology and KEGG pathways enrichment analysis showing the biological processes, cellular components, molecular functions and KEGG pathways which are associated with the T2DM proteins targeted by AK. Insulin resistance is the most enhanced KEGG pathway.

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Results and discussion

113 AK compounds, 59 with good oral bioavailability, 44 were found to have predicted targets; 760 total AK compounds targets; 512 T2DM associated genes

124 genes associated with T2DM targeted by AK compounds

In the present study, network pharmacology was utilized to determine the targets of AK compounds in T2DM. Through this method, it was identified that **TNF, STAT3, TP53, AKT1, EP300, PPARG and PTGS2** are the pivotal genes targeted by AK in T2DM. Moreover, molecular docking was conducted to determine the possible binding of AK compounds towards these identified targets.

Results and discussion

Table 1. Calculated binding energies of the different AK compounds when docked to the different pivotal proteins.

Protein	Ligand	Binding Energy (kcal/mol)
PPARG (PDB ID: 4ema)	(2E)-1-[4-hydroxy-2-(2-hydroxy-2-propanyl)-2,3-dihydro-1-benzofuran-7-yl]-3-(4-hydroxyphenyl)-2-propen-1-one	-8.7
	Dorsmannin A	-8.5
	deoxydihydroxanthoangelol H	-8.2
	deoxyxanthoangelol H	-8.2
	isobavachin	-8.1
	rosiglitazone[A]	-8.5
	15-Deoxy-PGJ2[A]	-7.7

[A]reference compound

Results and discussion

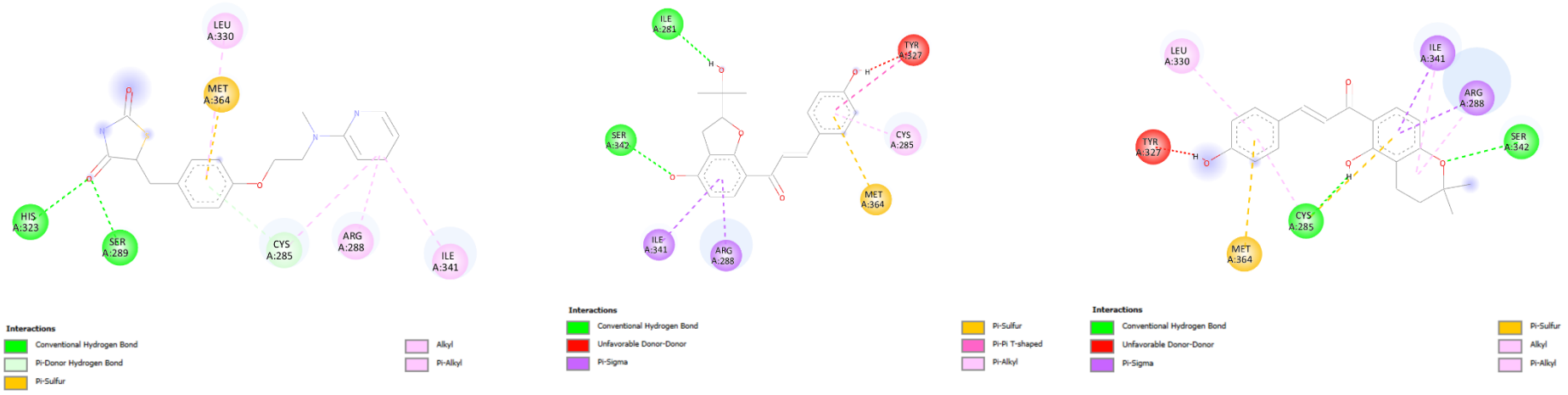

Figure 4A. Protein-ligand interactions between PPARG (PDB ID: 4ema) and rosiglitazone (left), (2E)-1-[4-hydroxy-2-(2-hydroxy-2-propanyl)-2,3-dihydro-1-benzofuran-7-yl]-3-(4-hydroxyphenyl)-2-propen-1-one (center) and dorsmannin A (right)

Results and discussion

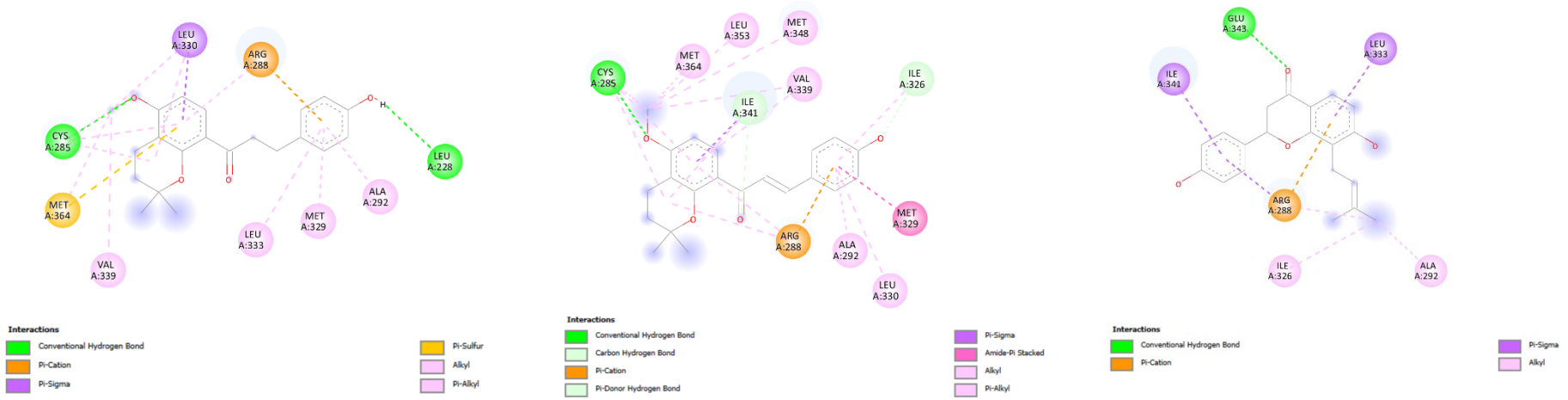

Figure 4B. Protein-ligand interactions between PPARG (PDB ID: 4ema) and deoxydihydroxanthoangelol H (left), deoxyxanthoangelol H (center), and isobavachin (right).

Results and discussion

In glucose control, the activating **PPAR-γ** results in the reduction in the production of glucose in the liver; **enhancement of glucose absorption** in the peripheral tissues by activating GLUT-4; decreased glucose-fatty acid cycle process and the reduction of deposition of ectopic lipids in muscle and liver

Mirza AZ, Althagafi II, Shamshad H. Role of PPAR receptor in different diseases and their ligands: Physiological importance and clinical implications. *European Journal of Medicinal Chemistry*. 2019;166:502-513. doi:10.1016/j.ejmech.2019.01.067

Results and discussion

Table 1. (cont.)

Protein	Ligand	Binding energy (kcal/mol)
TNFA (PDB ID: 2az5)	Isobavachin	-8.5
	307[A]	-9.1
TP53 (PDB ID: 3dcy)	Steviol	-8.5
	Pifithrin alpha[A]	-7.5
AKT1 (PDB ID: akt1)	deoxydihydroxanthoangelol H	-8.8
	4-Hydroxy-2',3'-(2,3-dihydro-2-hydroxy isopropylfurano)-4'-methoxychalcone	-8.3
	SM[A]	-10.4

[A]reference compound

Results and discussion

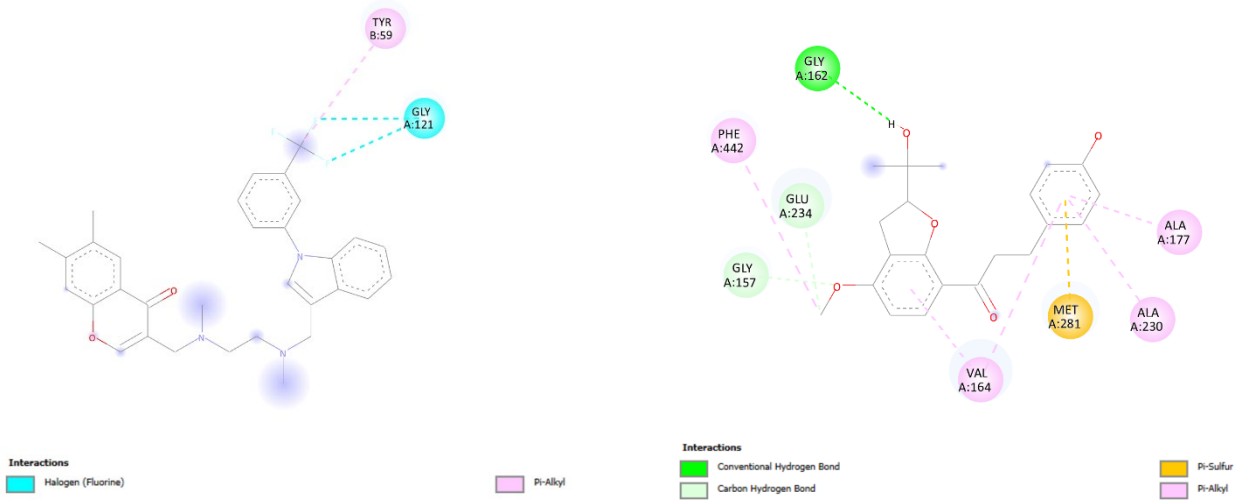

Figure 5. Protein-ligand interactions between TNFA (PDB ID: 2az5) and 307 (left) and isobavachin (right)

Results and discussion

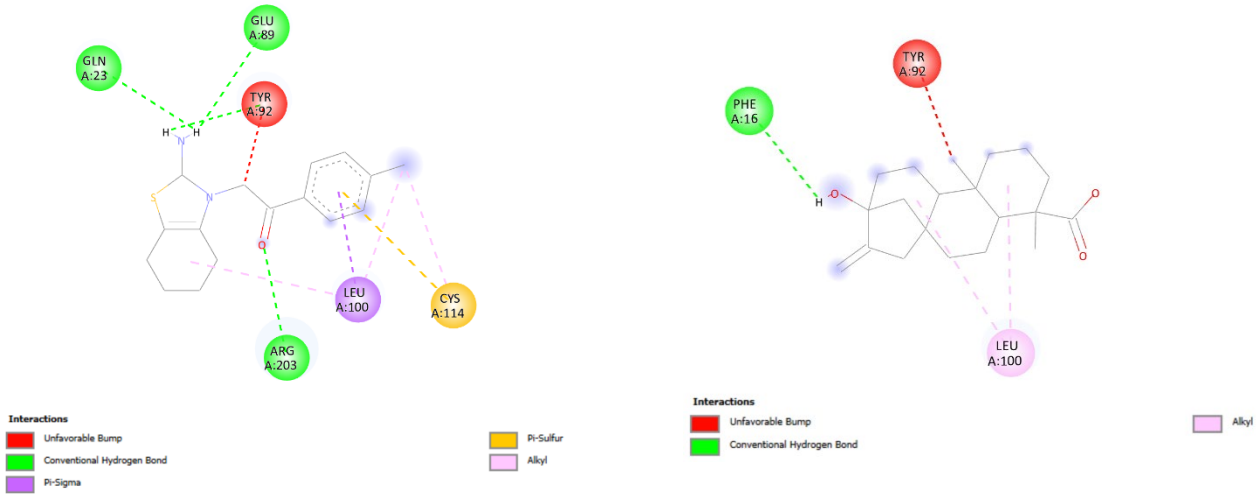

Figure 6. Protein-ligand interactions between TP53 (PDB ID: 3dcy) and pifithrin alpha (left), steviol (right)

Results and discussion

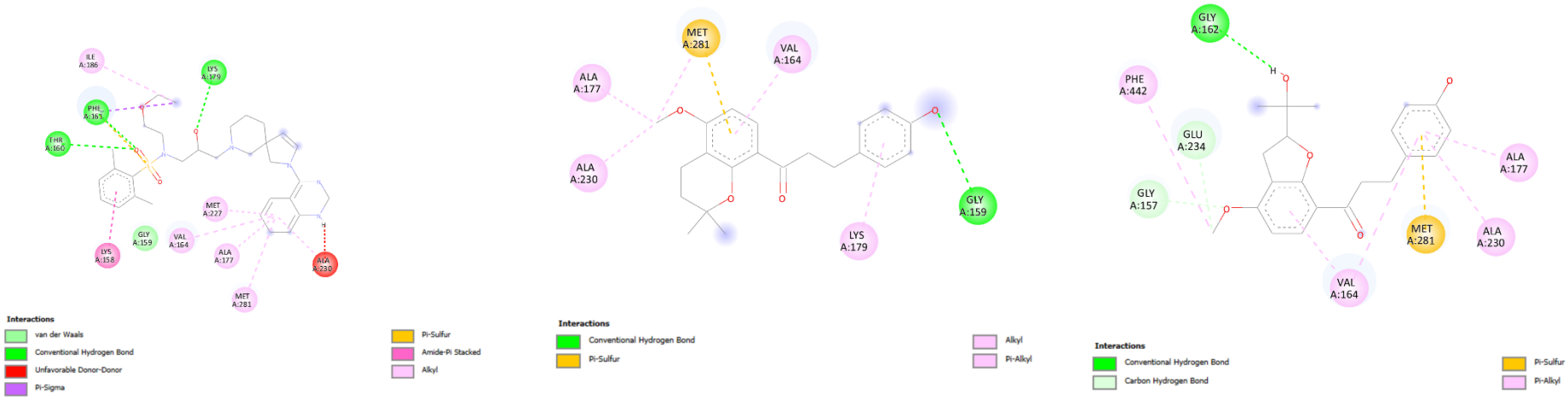

Figure 7. Protein-ligand interactions between AKT1 (PDB ID: 3qkm) and SM (left), deoxydihydroxanthoangelol H (center) and 4-Hydroxy-2',3'-(2,3-dihydro-2-hydroxy isopropylfurano)-4'-methoxychalcone (right)

Results and discussion

TNF-α → T2DM development (oxidative stress and inflammation); reduced GLUT-4 expression; apoptosis in β-cells; insulin resistance

P53 represses the expression of GLUT1 and GLUT4; has been implicated as one of the driving factors for tissue-specific insulin resistance

Activation of the **PI3K/AKT** pathway allows the intracellular uptake of glucose via the GLUT-4 in lipocytes, hepatocytes and muscle cells.

Gim HJ, Choi YS, Li H, Kim YJ, Ryu JH, Jeon R. Identification of a Novel PPAR-γ Agonist through a Scaffold Tuning Approach. *Int J Mol Sci*. 2018;19(10):3032. doi:10.3390/ijms19103032

Enoki T, Ohnogi H, Nagamine K, et al. Antidiabetic Activities of Chalcones Isolated from a Japanese Herb, *Angelica keiskei*. *J Agric Food Chem*. 2007;55(15):6013-6017. doi:10.1021/jf070720q

Itahana Y, Itahana K. Emerging Roles of p53 Family Members in Glucose Metabolism. *Int J Mol Sci*. 2018;19(3):776. doi:10.3390/ijms19030776

Rath S, Jagadeb M, Bhuyan R. Molecular docking of bioactive compounds derived from Moringa oleifera with p53 protein in the apoptosis pathway of oral squamous cell carcinoma. *Genomics Inform*. 2021;19(4):e46. doi:10.5808/gi.21062

Chen J, Xia Y, Sui X, et al. Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. *Oncotarget*. 2018;9(41):26299-26308. doi:10.18632/oncotarget.25233

Miao R, Fang X, Wei J, Wu H, Wang X, Tian J. Akt: A Potential Drug Target for Metabolic Syndrome. *Frontiers in Physiology*. 2022;13. Accessed August 22, 2022. https://www.frontiersin.org/articles/10.3389/fphys.2022.822333

Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathwway in obesity and type 2 diabetes. *Int J Biol Sci*. 2018;14(11):1483-1496. doi:10.7150/ijbs.27173

Results and discussion

Table 1. (cont.)

Protein	Ligand	Binding energy (kcal/mol)
PTGS2 (PDB ID: 5ikr)	Selidinin	-8.1
	Columbianadin	-8.1
	Mefenamic acid[A]	-9.1
EP300 (PDB ID: 7vhz)	deoxyxanthoangelol H	-10.6
	deoxydihydroxanthoangelol H	-9.8
	munduleaflavanone A	-9.6
	6TI[A]	-13.4
STAT3 (PDB ID: 1bg1)	xanthokeismin A	-8.3
	STX[A]	-10.7

[A]reference compound

Results and discussion

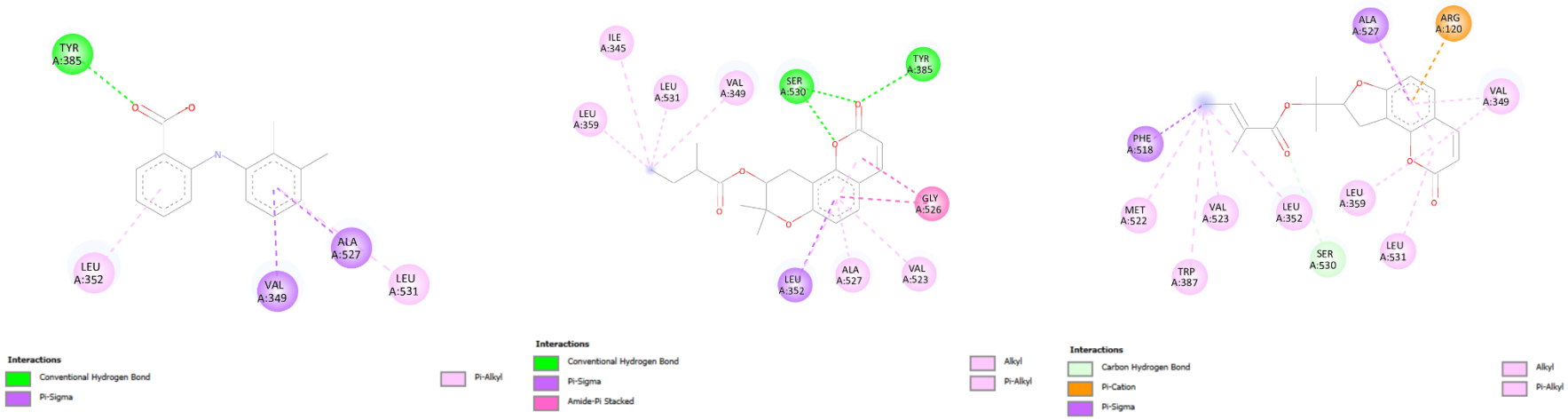

Figure 9. Protein-ligand interactions between PTGS2 (PDB ID: 5ikr) and mefenamic acid (left), selidinin (center) and columbianadin (right)

Results and discussion

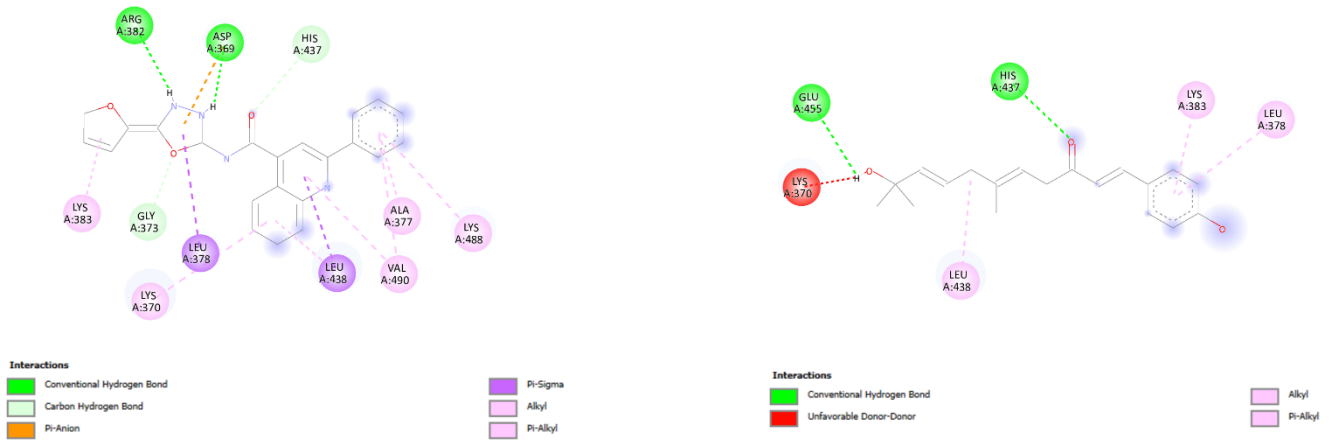

Figure 8. Protein-ligand interactions between STAT3 (PDB ID: 1bg1) and STX (left), and xanthokeismin A (right)

Results and discussion

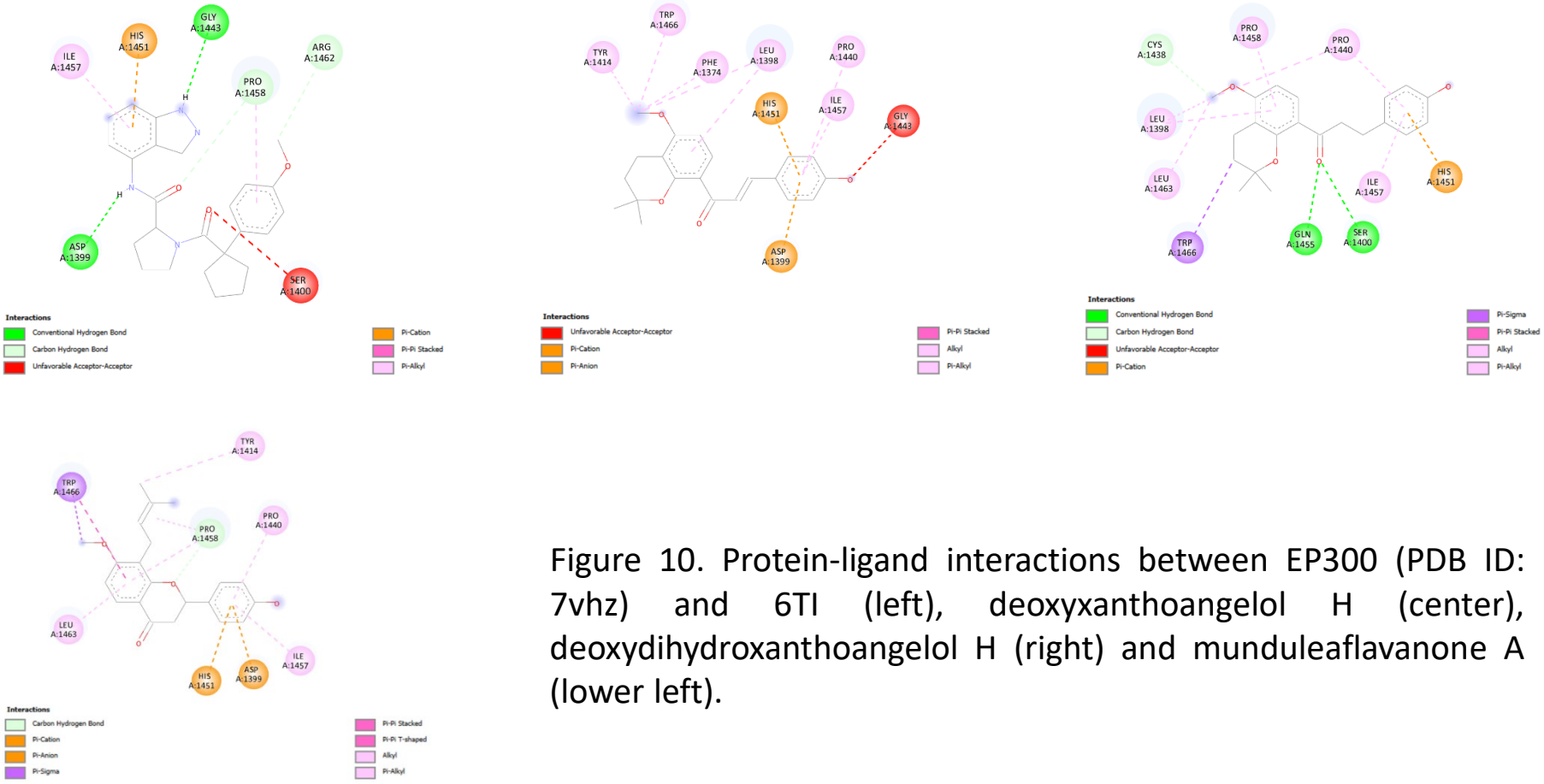

Figure 10. Protein-ligand interactions between EP300 (PDB ID: 7vhz) and 6TI (left), deoxyxanthoangelol H (center), deoxydihydroxanthoangelol H (right) and munduleaflavanone A (lower left).

Results and discussion

COX-2 (PTGS2) mediated inflammation contributes to complications of metabolic syndrome and diabetes including insulin resistance. They are crucially involved in the development of metabolic syndrome associated with obesity and are also involved in the regulation of energy metabolism under pathologic conditions.

The levels of **p300** in β-cells are downregulated and decreased in animal model predisposed to developing diabetes. Moreover, in the simulation of hyperglycemia and also of inflammation in INS-1E cells, there was an observed decrease in the levels of p300 protein content.

Inhibition of **STAT3** has been shown to reprogram non-β cells into insulin producing cells and increased in the number of islet-like clusters in the pancreas

Oniga SD, Pacureanu L, Stoica CI, et al. COX Inhibition Profile and Molecular Docking Studies of Some 2-(Trimethoxyphenyl)-Thiazoles. *Molecules*. 2017;22(9):1507. doi:10.3390/molecules22091507

Puratchikody A, Sriram D, Umamaheswari A, Irfan N. 3-D structural interactions and quantitative structural toxicity studies of tyrosine derivatives intended for safe potent inflammation treatment. *Chemistry Central Journal*. 2016;10(1):24. doi:10.1186/s13065-016-0169-9

Menghini L, Epifano F, Genovese S, Marcotullio MC, Sosa S, Tubaro A. Antiinflammatory activity of coumarins from Ligusticum lucidum Mill. subsp. cuneifolium (Guss.) Tammaro (Apiaceae). *Phytother Res*. 2010;24(11):1697-1699. doi:10.1002/ptr.3170

Jayakumar T, Hou SM, Chang CC, et al. Columbianadin Dampens In Vitro Inflammatory Actions and Inhibits Liver Injury via Inhibition of NF-κB/MAPKs: Impacts on ·OH Radicals and HO-1 Expression. *Antioxidants*. 2021;10(4):553. doi:10.3390/antiox10040553

Ruiz L, Gurlo T, Ravier MA, et al. Proteasomal degradation of the histone acetyl transferase p300 contributes to beta-cell injury in a diabetes environment. *Cell Death Dis*. 2018;9(6):1-12. doi:10.1038/s41419-018-0603-0

Lan F, Hu Y, Tang D, Cai J, Zhang Q. Transcription coactivator p300 promotes inflammation by enhancing p65 subunit activation in type 2 diabetes nephropathy. *Int J Clin Exp Pathol*. 2019;12(5):1826-1834.

Mashili F, Chibalin AV, Krook A, Zierath JR. Constitutive STAT3 Phosphorylation Contributes to Skeletal Muscle Insulin Resistance in Type 2 Diabetes. *Diabetes*. 2013;62(2):457-465. doi:10.2337/db12-0337

Miura M, Miyatsuka T, Katahira T, et al. Suppression of STAT3 signaling promotes cellular reprogramming into insulin-producing cells induced by defined transcription factors. *EBioMedicine*. 2018;36:358-366. doi:10.1016/j.ebiom.2018.09.035

Results and discussion

Compounds found in ashitaba show favorable binding towards the pivotal proteins as shown by the negative binding affinity values and the similarity in the amino acid residues present in their protein ligand interactions and that of the protein and the reference compounds.

Favorable binding towards the pivotal proteins suggests that ashitaba compounds can potentially help as an adjunct for conventional treatments. However, we are bound to the limitations of computational methods and further experimentation are needed to validate our results

Oniga SD, Pacureanu L, Stoica CI, et al. COX Inhibition Profile and Molecular Docking Studies of Some 2-(Trimethoxyphenyl)-Thiazoles. *Molecules*. 2017;22(9):1507. doi:10.3390/molecules22091507

Puratchikody A, Sriram D, Umamaheswari A, Irfan N. 3-D structural interactions and quantitative structural toxicity studies of tyrosine derivatives intended for safe potent inflammation treatment. *Chemistry Central Journal*. 2016;10(1):24. doi:10.1186/s13065-016-0169-9

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Conclusions

- Pivotal genes in T2DM that are targeted by the reported ashitaba compounds include TNF, STAT3, TP53, AKT1, EP300, PPARG and PTGS2 which code for the proteins TNF-α, STAT3, p53, AKT1, HAT p300, PPAR-γ and COX-2, respectively.

- Compounds that are present in ashitaba which target these proteins include, deoxyxanthoangelol H, munduleaflavanone A, deoxydihydroxanthoangelol H, (2E)-1-[4-hydroxy-2-(2-hydroxy-2-propanyl)-2,3-dihydro-1-benzofuran-7-yl]-3-(4-hydroxyphenyl)-2-propen-1-one, dorsmannin A, isobavachin, selidinin, columbianadin, xanthokeismin A, isobavachin, steviol, and 4-Hydroxy-2',3'-(2,3-dihydro-2-hydroxyisopropylfurano)-4'-methoxychalcone.

Conclusions

- Experimental validation must be conducted since most of these compounds have not been reported to act on the pivotal targets.

- The results of this study give insights on the possible biomolecular mechanisms of ashitaba compounds toward T2DM hence rationalizing its possible therapeutic effect; and giving insights on the possible synergism or antagonism when it is taken with drugs used in the management and treatment of the disease.