
**Arab American University**

**Faculty of Graduate Studies**

# In silico insights into natural ligands affecting key proteins in the insulin signaling pathway: AS160 and PTEN.

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**This thesis was submitted in partial fulfillment of requirements for the Master's degree in Cellular and Molecular Bio-sciences**

**October /2023**

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# Thesis Approval

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This thesis was defended successfully on the October 10th; 2023 and approved by:

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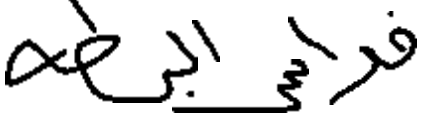
| 3. Dr. Feras Fayez Abdellatif Al Battah - Internal Examiner : |  |
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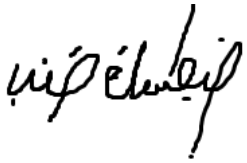
## Declaration

I certify that the work provided in this thesis, unless otherwise referenced, is the researchers work and has not been submitted for a higher degree to any other university or institution.

Shahid Hassan Amin Abu-Naim

Signature:  .

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## Dedication

I dedicate this effort to those who are not matched by anyone in the universe, to whom God has commanded us to honor them, to those who have made a great deal, and have given what cannot be returned, to you these words, my beloved mother and dear father:

"Nadia Mallah & Hassan AbuNaim"

I dedicate this research to you; you have been my best supporter .throughout my academic career.

To the students of science and the researchers the former and subsequent of them, to the candles that are burning to show us the way, to the professors and researchers in the world, to all who have contributed to my support and encouragement, to all who have helped me.

## Acknowledgments

I thank God Almighty first and foremost for the great grace that He has bestowed upon me, For everyone who advised me, guided me, contributed, or directed me with me in preparing this research and connecting me to the required references and sources at any of the stages it went through, and I especially thank the distinguished doctors:

“Dr. Siba Shanak” and "Prof. Hilal Zaid"

for helping me, supporting me and guiding me with advice, education, correction, and all that they did with me.

I am also pleased to thank the esteemed college administration: "Arab American University – Palestine, Health and science department"

## Abstract

Insulin is a crucial hormone that regulates blood sugar levels. It is secreted post-prandial and binds to insulin receptors in liver, muscle, and fat cells. This leads to uptake of glucose in the form of glycogen or fat, a process that involves multiple proteins and enzymes, including PTEN and AS160, which are regulatory proteins in the insulin pathway and inhibit the insulin cascade. Deficits in the insulin signal pathway lead to insulin resistance and, eventually, type 2 diabetes mellitus. In natural compounds extracted from plant species, such as D-talose, inositol, glucopyranose, and alpha-linolenic acid, have been reported as anti-diabetic agents. This *in silico* study reports potential natural chemical inhibitors for two main protein inhibitors in the insulin signaling pathway, PTEN and AS160, and as such potential treatment of diabetes mellitus. Therefore, we aim to predict the drug-likeness potential of 17 natural phytochemicals from *Gundelia tournefortii* and *Ocimum* to treat diabetes. This is achieved by testing their interaction (the maximum binding affinity and the interacting amino acid residues) with AS160 and PTEN *in silico*, using Autodock tools. Then we identified their drug-likeness and ADME (absorption, distribution, metabolism and excretion) properties using swissADME web tool where all the selected ligands were screened based on the Lipinski's rule of five to find the drug likeness and other properties using the Bioavailability Radar.

Several compounds have shown high binding affinities with both target protein e.g., beta-sitosterol, beta-amyrin, lupeol-trifluoroacetate, lupeol and Stigmasterol. For ADME analysis, the study analyzed 17 compounds, following Lipinski's rule of five, with the best bioavailability found in stearic-acid, palmitic-acid, ferulic-acid, isovanillic-acid, 4-methoxybenzoic-acid, and 4-hydroxybenzoic-acid. However, 3,4-dihydroxybenzoic-acid bioactive compounds show good solubility but not specificity; they react nonspecifically with numerous biological targets rather

than specifically affecting one desired target- and an inhibitor of Cytochromes P450 (CYP enzymes). Beta-sitosterol, beta-Amyrin, and lupeol-trifluoroacetate show poor solubility and no excretion problems. The results indicated that five out of 17 tested ligands bind well with both target proteins; PTEN and AS160 based on their high binding affinity. Taking this together, besides their drug-likeness properties, they can be potent antidiabetic drugs to be tested *in vitro* and *in vivo* systems.

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## 1. Introduction

Diabetes is a group of diseases characterized by high levels of glucose in the blood resulting from defects in insulin production (insulin deficiency), insulin action (insulin resistance), or both. Two main types of diabetes are known: Type 1 diabetes (TID) and Type 2 diabetes (T2D).

T1D is called insulin-dependent diabetes or juvenile-onset diabetes. It develops when the body's immune system destroys pancreatic beta-cells, the only cells that produce insulin. The human system, in turn, produces little or no insulin, resulting in insulin deficiency (Association, 2009). T2D is also called non-insulin-dependent or adult-onset diabetes. Insulin resistance, which is characterized by a decreased sensitivity of body cells to the signals of insulin, is what causes the disease to start. As the condition progresses, the ability to make insulin gradually declines over time (Olokoba et al., 2012). High blood sugar (glucose) is a symptom of gestational diabetes, which normally disappears after delivery. T2D risks are higher for pregnant women who have gestational diabetes (Dirar & Doupis, 2017; Phelan et al., 2021).

Insulin is a polypeptide pancreatic hormone that plays an essential role in regulating blood glucose. In reaction to elevated blood glucose levels, insulin is quickly released. Different organs or "stations" along insulin's physiological journey are meticulously regulated for insulin manufacture, quality control, distribution, and activity (Omar-Hmeadi & Idevall-Hagren, 2020). The hormone insulin must first be transcribed and expressed in the pancreatic beta-cells in order for it to begin acting. Insulin is then exported from the body through the portal circulation to the liver, where the liver's hepatocytes clear about 50% of the insulin on the first pass. The remaining insulin leaves the liver through the hepatic vein and travels through the venous system to the heart and the rest of the body. Insulin that has been injected into the blood vessels works on metabolism in the liver before being further eliminated (second pass). At the level of the

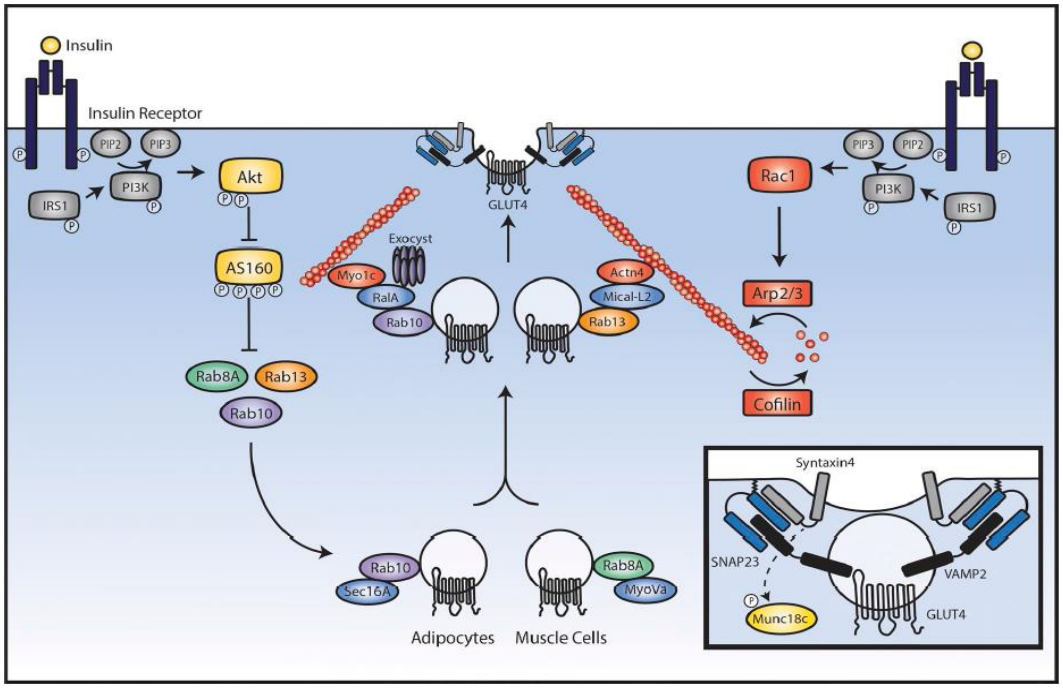
microvasculature, insulin leaves the bloodstream and enters muscle and fat cells, where it promotes glucose transporter 4 (GLUT4) translocation and glucose absorption. The final step in the action of remaining circulating insulin is its degradation in the kidney (Tokarz et al., 2018).

The challenge is to find drugs for diabetes rather than insulin, these drugs targeting certain protein in the insulin signaling pathway using phytochemicals that have positive effect in glucose uptake

## 1.1 insulin signaling pathway: AS160 and PTEN proteins

Insulin signaling in muscle and adipose cells leads to the recruitment of glucose transporter-4 (GLUT4) to the plasma membrane, which allows glucose uptake for metabolism and glycogen synthesis. The process begins when Insulin binds to its receptor (IR) on the surface of muscle or fat cells and activates the IR tyrosine kinase activity toward autophosphorylation by inducing structural rearrangement of the transmembrane domains to bring them into close proximity with each other, and the consequent activation of the IR tyrosine kinase toward phosphorylation of its major substrates IRS1, 2. Phosphorylation sites on IRS1,2 constitute entropic information to attract class I Phosphoinositide 3-kinases (PI3K), which rapidly generates membrane domains enriched in Phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 attracts the PH domain of Akt, which makes the protein available for phosphorylation. Activated Akt1,2 migrates to the cytosol and intracellular membranes (Zheng & Cartee, 2016), where it phosphorylates *AS160*, a substrate of 160 kD more appropriately named TBC1D4. (TBC1- Tre-2, BUB2, CDC16- Domain Family Member 4) The TBC domain of AS160/TBC1D4 defines its GAP (GTPase-activating proteins) activity toward Rab(Ras-associated binding) family small GTPases(hydrolase enzymes that bind to the nucleotide guanosine triphosphate (GTP). Phosphorylation of AS160 inhibits its GAP

activity; hence, insulin signaling leads to inactivation of an inhibitor of Rab GTPases. It is indeed important to regulate vesicle traffic in the pathway. Herein, Rab GTPases regulate vesicle fission, destination, and fusion. AS160/TBC1D4 targets a cluster of Rabs, particularly the phylogenetically related Rabs 8A, 10, and 13. In addition to AS160, *PTEN*, Phosphatase and TENsin homolog is another regulatory protein found in the insulin cascade where it functions as a phosphatase. In humans, the protein is encoded by the PTEN gene. The protein contains a tensin-like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate. It also inactivates the IR1.2, leading to the inhibition of the insulin signaling pathway, Figure 1 (Tokarz et al., 2018)Inhibition of the insulin-signaling pathway leads to insulin resistance, an early step in the development of T2D.



In parallel to the activation of "Akt cascade," in insulin signaling pathway, the burst in plasma membrane–associated PIP3 leads to the activation of Rac1 (Takenaka et al., 2016). The resulting Rac1 activation leads to a dynamic remodeling of cortical actin filaments via cycles of actin filament branching enacted by Arp2/3 and actin severing enacted by cofilin, which is best mapped in muscle cells (Chiu et al., 2010). In adipocytes, Rab10 is the preferred GTPase in GLUT4 translocation, whereas in muscle cells, it is Rab8A and Rab13. In adipocytes, Rab10 promotes GLUT4 mobilization from the perinuclear region toward the plasma membrane (Sano et al., 2007, Bruno et al., 2016), specifically by interacting with Sec16A (Bruno et al., 2016). In muscle cells, Rab8A engages its effector Myosin Va thereby promoting GLUT4 exit from the storage compartment (Sun et al., 2014). This processive molecular motor allows the migration of GLUT4 vesicles along actin filaments toward the cell periphery. Rab13 is more peripherally located, and its effector is the cortically located protein MIC AL-L2, which in turn binds the cortical cytoskeletal protein α-actinin4. In response to insulin, these three proteins can be visualized near the cell surface along with GLUT4 and cortical actin (Sun et al., 2016). In this way, Rab8A and Rab13 ensure GLUT4 vesicle mobilization toward the periphery and GLUT4 vesicle being ready to fuse with the plasma membrane (Yehia et al., 2019). .The chemical compounds that have been experimentally reported to have a positive effect in GLUT4 translocation- enhanced GLUT4 translocation- (Kadan et al., 2018) are: 3,4-Dihydroxybenzoic_acid, 4-Methoxybenzoic acid, beta-sitosterol, beta-Amyrin, Chlorogenic acid, Isovanillic acid, linalool, lupeol-trifluoroacetate, lupeol, myo-Inositol, stigmasterol, palmitic acid, stearic acid, caffeic_acid, D-pintol, ferulic acid, and 4-hydroxybenzoicacid, their 2d structures shown in Fig.2. These phytochemicals were extracted from the two medical plants;

*Gundelia tournefortii* and *Ocimum basilicum* that have been reported as an anti-diabetic herbs (Kadan et al., 2018, Kadan et al., 2021).

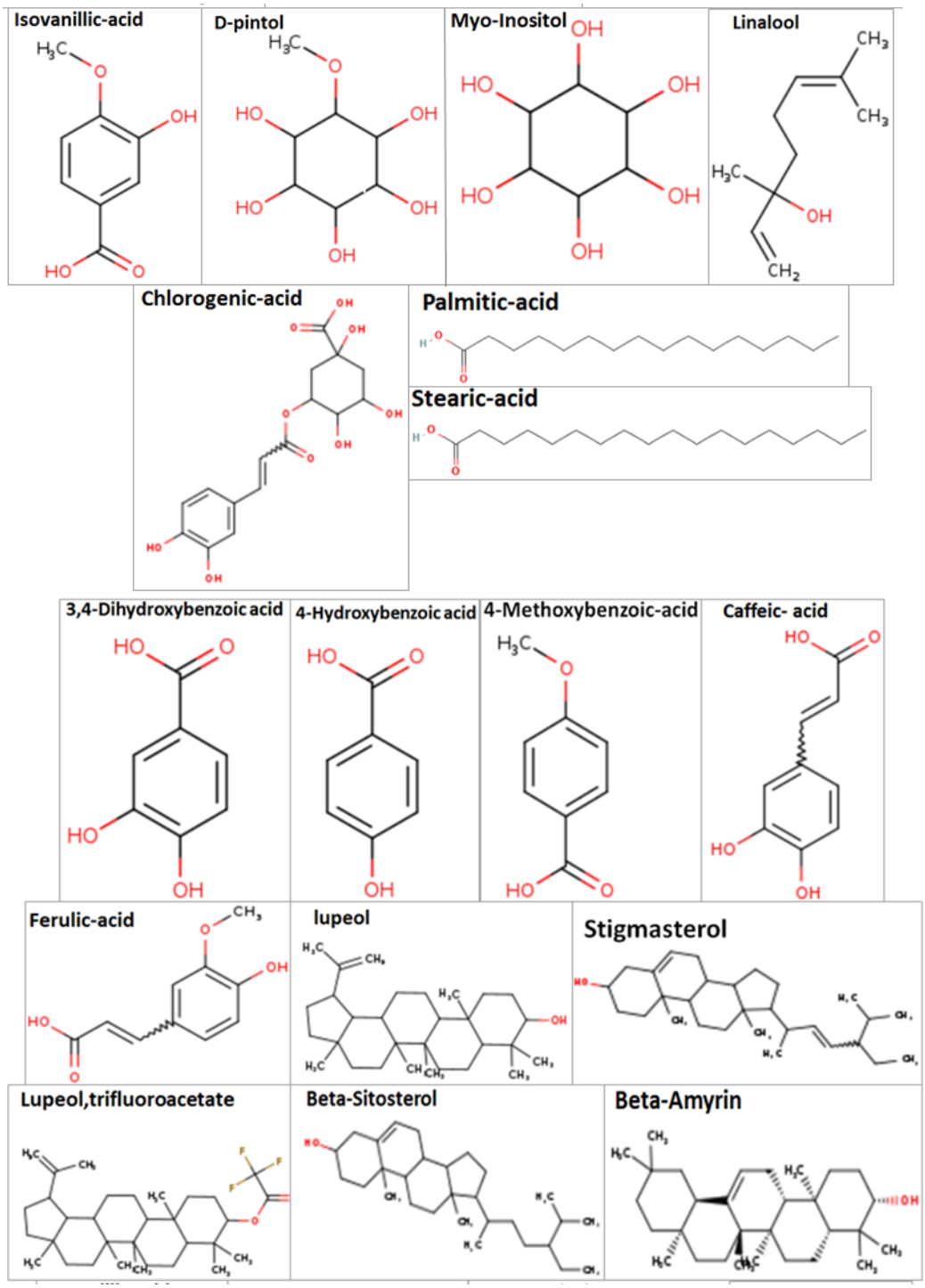



Figure (2): Chemical structures of the compounds found in *Gundelia tournefortii* and *Ocimum basilicum* and that has a positive effect in GLUT4 translocation.

## 1.2 Medicinal plants

Medicinal plants are increasingly used as sources of bioactive compounds required for drug development (Mrabti et al., 2021). The World Health Organization (WHO) estimates that approximately 80% of the population in developing countries depends on medicinal plants and traditional medicine for their primary health care estimated by the World Health Organization (WHO) (Joint & Additives, 1989). Many modern pharmaceutical researchers continue to focus on the discovery and evaluation of natural compounds for possible therapies for obesity, diabetes, infections, cancer, and oxidative stress.

*Gundelia tournefortii* grows in the mountainous, tropical, or temperate region. This plant is most widely distributed in the Mediterranean Sea region, African nations, the Middle East, Afghanistan, Turkmenistan, and areas beyond the Caucasus. The Asteraceae, which has more than 13,000 species and 900 genera, is the biggest family of flowering plants. Iran only includes one species of the Asteraceae genus *Gundelia,* known as *the Gundelia tournefortii* (Karimzadeh et al., 2023). A perennial, robust, and succulent plant in the genus *Gundelia* has alternate leaves and pinnate divisions with serrated sides that grow into thorns. In diabetic mice, the aqueous root, shoot, and aerial component extracts all showed a measurable hypoglycemic effect. The Mediterranean Sea region, African nations, and the Middle East are considered to have the highest distribution of this plant on a global scale. The number of islets of Langerhans and their average width may increase, decreasing the amount of glucose absorbed from the intestine and raising insulin sensitivity are some of the potential mechanisms of antidiabetic activity.(Moghaddam, 1998).

*Ocimum basilicum,* also known as Basil or Rehan, includes about 150 species – a famous culinary herb, also called sweet basil, which is a universally cultivated, perennial herbaceous

plant(Eid et al., 2023). *Ocimum basilicum* is a fragrant medicinal herb that grows all over the world and is primarily utilized in traditional medicine. This plant is utilised as a kitchen herb, a culinary herb, and a decorative herb (Gülçin et al., 2007). *O. basilicum* is undoubtedly one of Morocco's most significant medicinal herbs. In actuality, it is an essential oil-producing plant that can grow in a variety of environments and on a variety of surfaces. Basil has historically been used in Morocco to cure a variety of illnesses and conditions, including diabetes, infectious infections, sinusitis, tachycardia, haemorrhoids, and inflammation when consumed as a herbal tea (Joshi, 2014). This plant has been utilized in different nations to treat conditions like headaches, colds, bug bites, nerve discomfort, kidney ailments, and cardiovascular diseases. Several articles have investigated the biological properties of *O. basilicum*, including antioxidant (Teofilović et al., 2021), anti-microbial anticancer (Dolghi et al., 2021), immunomodulatory, insecticide, anti-thrombotic, antiplatelet, anti-inflammatory, anti-hyperlipidemic, and anticonvulsant effects (Qasem et al., 2023). Other investigations reveal that the extract of *O. basilicum's* aerial portions had a stronger hypoglycemic impact. It promoted glucose mobilization by promoting hepatic glycogen synthesis, increased liver glycogen content, and enhanced oral glucose tolerance. *In vitro* tests were performed to determine whether extracts from *O. basilicum's* aerial parts had any anti-diabetic effects. In particular, the methanol and hexane extracts enhanced the translocation of the glucose transporter to the plasma membrane (Kadan et al., 2016).

### 1.3 Computational modeling

Using computer modeling cuts down the research timeline and cost, and it also reduces wet-lab experiment, as the drug discovery takes years to decades for discovering a new drug and is very costly(Chopra & Samudrala, 2016). Molecular docking is a computational technique used in

molecular biology and computer-aided drug design to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. The goal of molecular docking is to find the best orientation of a ligand in the binding site of a protein, which can be used to predict the strength of association or binding affinity between two molecules (Pinzi & Rastelli, 2019). This facilitates the identification of novel therapeutics at the molecular level.

There are two types of docking 1) Rigid Docking, which is identified as the lock and key model; where the internal geometry of both the receptor (target) and ligand are treated as rigid. 2) Flexible docking (induced fit), where the receptor and ligand have to change their conformation to fit each other well (Chen et al., 2020). The main key stages of docking (Torres et al., 2019) are: protein and ligand selection, protein and ligand preparation, molecular docking and evaluating docking results. The type is used in this study is that the ligand is only flexible while the receptor treated as rigid.

Successful docking methods use a scoring function that correctly ranks candidate dockings and search high-dimensional spaces effectively. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site (Ravindranath et al., 2015). There are multiple widely used and effective docking programs that employ different strategies for sampling ligand orientations and conformations in the protein binding site (Bender et al., 2021). A process that often involves sampling hundreds-of-thousands to millions of compounds (Meng et al., 2011) .

For protein selection, the protein-ligand docking assay requires the structure of a target protein. Of total, 87% of those available in the most comprehensive resource, the Protein Databank

(PDB; http://www.rcsb.org/pdb/) is the single worldwide archive of structural data of biological macromolecules, where the protein structures were deposited in the PDB by varying expertise in the techniques of X-ray crystal structure determination, NMR, cryoelectron microscopy and theoretical modeling (Berman et al., 2003, Berman et al., 2002). Most of these structures usually do not meet the quality criteria required for a modeling study. For instance, some atoms in the flexible part of the protein are not resolved and the corresponding spatial coordinates are omitted in the PDB file (Berman, 2008)

The protein-ligand docking assay also requires the structure of ligand, and it can be obtained from small molecule databases such as PubChem (Torres et al., 2019). PubChem is a database of chemical molecules and their activities against biological assays (https://pubchem.ncbi.nlm.nih.gov/). It also provides a wealth of information on chemical substances, including their structures, properties, and biological activities. More than 400 data contributors have provided descriptions of chemical substances and information about biological activity to PubChem (Kim et al., 2016) (Wang et al., 2014). Thus it is a vital resource for the biomedical research communities in many fields, including drug development. Although the majority of the information in PubChem is on small molecules, it also has information on other types of molecules, including peptides, lipids, carbohydrates, small-interfering and micro-RNAs (siRNAs and miRNAs) (Xie, 2010). Because the configuration of the protein structure and the ligand requires an adequate grasp of both organic and/or medicinal chemistry and biophysics, docking software is sometimes inaccessible to a large audience outside of molecular modelling specialists (Grosdidier et al., 2011, Pinzi & Rastelli, 2019).

Following the configuration of the two structures, a number of docking parameters must be determined, such as the area of space to which the docking search space will be constrained, the

thoroughness of the conformational search, or the maximum duration allowed for the docking assay. The majority of docking programs are complex computational tools that need unique additional sampling or scoring settings to which they may be extremely sensitive (Grosdidier et al., 2011).

Docking programs are widely used to identify drug-like molecules interacting with a given receptor to inhibit its function. Most docking programs model small molecules as flexible while modeling receptors as rigid, which limits the range of therapeutic targets for which docking can be applied. However, there are alternative approaches that incorporate receptor flexibility in docking simulations:

- Flexible ligand docking to multiple receptor conformations: Fully receptor flexible docking can be performed to force the receptor into alternative conformations, allowing for more flexibility in the docking process (Totrov & Abagyan, 2008).
- Consideration of receptor flexibility: Some docking methods incorporate receptor flexibility by using an ensemble of precompiled receptor conformations or by enlarging the binding pocket. This approach has been reported to be useful in improving docking accuracy (Feixas et al., 2014) .
- Interactive docking and virtual reality: Interactive docking methods, where the binding site is already known, can be used for flexible receptor docking. This approach allows for real-time manipulation of the receptor conformation in virtual reality, enhancing the understanding of ligand-receptor interactions (Iakovou et al., 2022).
- Treatment of molecular flexibility: With the increase in computing power, flexible-ligand docking has become a standard protocol in protein-ligand docking. However, rigid

proteins are still widely used in real applications due to the computational complexity of considering receptor flexibility (Forrey et al., 2012).

- Docking programs with receptor flexibility: Several docking programs have been developed that explicitly consider receptor flexibility. For example, AutoDockFR simulates partial receptor flexibility by allowing explicitly specified receptor side-chains to explore their conformational space(Ravindranath et al., 2015). ReFlexIn represents a flexible receptor by a series of potential grids corresponding to discrete receptor conformations(Leis & Zacharias, 2012).

Even though that there are alternative approaches that incorporate receptor flexibility which allow for a wider range of therapeutic targets and improve the accuracy of docking simulations. However, many docking programs still model receptors as rigid such as AutoDockTools (ADT). AutoDockTools is software used for molecular docking simulations. It is a graphical user interface (GUI) that is part of the AutoDock suite of automated docking tools, It allows users to set up and run AutoDock (Goodsell et al., 2021). All components of the AutoDock suite are available under open source licenses and accessible through the AutoDock website (https://autodocksuite.scripps.edu). The program predicts the binding affinity of a small molecule to a target protein allowing users can view molecules in 3D, rotate and scale them in real-time, assign partial atomic charges to the ligand and macromolecule, set up rotatable bonds in the ligand. With a series of functions that walk the user through protonation, calculating charges, and identifying rotatable bonds in the ligand and the protein. AutoDockTools makes it easier to format input molecular files. Several cutting-edge techniques are included in AutoDockTools for grouping, visualising, and evaluating the outcomes of docking operations (Goodsell et al., 2021). for example a molecular viewer built on Python is available for free use called the Python

Molecular Viewer (PMV). The AutoDockTools dynamically extend PMV with instructions for setting up, initiating, and analyzing AutoDock calculations. As a result, AutoDockTools automatically supports all PMV functions, including reading and writing files, computing and showing secondary structure, adding and removing hydrogens, computing charges and molecular surfaces, and many others. A specialisation of the standard molecular viewer PMV for the particular AutoDock application is AutoDockTools (Morris et al., 2009).

When using AutoDockTools, parameters need to be specified to define various aspects of the docking process. AutodockTools parameters include:

- Grid: The parameter file for AutoGrid specifies the grid types, location, and dimensions. The grid is used to calculate the interaction energies between the ligand and the protein(Huey & Morris, 2006) .
- Docking: The parameter file for AutoDock specifies the molecules to be docked, the grids to be used, the algorithm to be used, and other relevant settings(Huey & Morris, 2006) .
- Output detail: The level of output detail in AutoDock can be controlled by the "outlev" parameter in the docking parameter file(Huey & Morris, 2008).

Atom parametrization: Autodock Tools provides pre-defined parameters for common atoms and molecules. However, if you need to parametrize an atom that is not included in the default parameters, you may need to manually define the parameters for that atom (Norgan et al., 2011).

The ability to specify and write command files for AutoDock Suit has been implemented by Chimaera (Goddard et al., 2018), PyMOL (Seeliger & de Groot, 2010), and many other third-party programs, offering non-experts a turnkey method to docking (Goodsell et al., 2021).

The PyMOL molecular graphics system has developed over the past few years from being a powerful molecular viewer with remarkable 3D capabilities into a platform for many programs and applications that utilize PyMOL's flexible visualization features (Seeliger & de Groot, 2010). It is a molecular visualization system (is a molecular modeling package found on-line https://pymolwiki.org/index.php/PLoS) that can produce high-quality 3D images of small molecules and biological macromolecules, such as proteins. It was created by Warren Lyford DeLano and was initially commercialized by DeLano Scientific LLC, which was a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities (Sridhar et al., 2017). PyMOL is open source but proprietary, and it is available for academic, non-profit, government, and commercial applications. It offers the flexibility of Python-based development and scalability. PyMOL can produce high-quality 3D images of small molecules and biological macromolecules. It supports over 30 different file formats and is available on Windows, Mac, and Linux (Dilip et al., 2016). Hundreds of parameters in PyMOL provide precise control over the appearance of structures. These parameters include options for controlling the color, transparency, and style of different parts of the structure, as well as options for adding labels, annotations, and other visual elements (DeLano & Bromberg, 2004). The precise control over the appearance of structures in PyMOL is achieved through the use of these parameters, which can be adjusted manually or through the use of Python scripts (Mooers, 2020)**.** This allows users to create highly customized images that highlight specific features of the structure they are studying (Yuan et al., 2017).

In order to determine a drug's safety and toxicity, researchers must look at how it acts in the body. An important phase in this procedure is the study of drug metabolism and pharmacokinetics, including toxicology and ADME investigations (Lai et al., 2022). These

investigations aim to evaluate the drug's efficacy by ascertaining if it can reach its target in the body in an adequate quantity and remain there in a bioactive form for the anticipated biologic events to take place (Ohtsuki et al., 2011).

ADME stands for Absorption, Distribution, Metabolism, and Excretion, which are the physiological procedures that define how a medication travels through and is metabolized by the body. Early in the development of a drug, pharmacokinetics studies enable the assessment of ADME features. Additionally, they offer crucial details about how food interactions, drug-drug interactions, and organ dysfunction affect the disposition of a drug (in the case of medications taken orally) (Tibbitts et al., 2016). For use in situations requiring a compromise between precision and speed, such as drug discovery and medicinal chemistry, the SwissADME tool was created (Daina et al., 2017), which is a free online application that gives users access to a collection of models that predict small molecule physicochemical characteristics, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness (Tibbitts et al., 2016). SwissADME is a user-friendly interface that makes data entry and result interpretation quick, simple, and effective (Daina et al., 2017).

## 1.4 Aims of the study

We aim in this study to predict the drug likeness of selected 17 phytochemical compounds - as potential drugs in fighting insulin resistance using molecular docking. This way, we might shed the light on identifying, these chemical compounds as inhibitors for hub proteins in the insulin signaling cascade: AS160 and PTEN, making them inactive. The protein- ligand docking has been processed between the ligands with each one of the key protein targets; AS160 and PTEN, found in the insulin cascade in muscle. The best docking results should have: higher binding free energy (Kcal/mol), lower inhibition constant (Ki), and lower RMSD (the Root-Mean-Square

Deviation of atomic positions) for the ligand from reference structure because high binding affinity point to stable binding. The study also evaluates the drug-likeness potential of the 17 compounds using SwissADME tool.

## 2. Materials & Methods:

### 2.1. Protein preparation

The three-dimensional structures of proteins (AS160 and PTEN) were be retrieved from the Protein Data Bank in RCSB (Research Collaboratory for Structural Bioinformatics) databank in PDB format as shown in Fig. 3. Protein data bank (Berman et al., 2000) which is available for free under an open-source license from https://www.rcsb.org/. Prior to molecular docking, protein is prepared by using AutoDock Tools software; (ADT is being distributed free of charge as part of the MGLTools package, at the WWW site: http://mgltools.scripps.edu/downloads. During preparation, polar hydrogens were added, and water molecules as well as hetero-atoms will be removed from the protein crystal structure for the prevention of unwanted interaction while docking (Zheng et al., 2019).

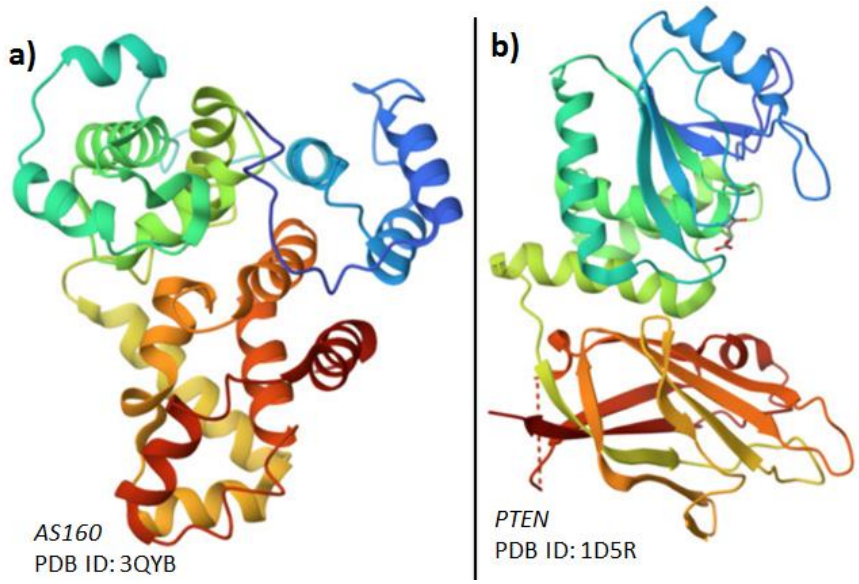



Figure (3): The 3D ribbon structures representation of target proteins visualized using RSCB PDB web site a) AS160 and b) PTEN.

## 2.2. Ligand preparation

The chemical structure of different chemical compounds: (3,4-dihydroxybenzoic acid, 4-methoxybenzoic acid, beta-sitosterol, beta-amyrin , Chlorogenic acid, Isovanillic acid, linalool, lupeol-trifluoroacetate, lupeol, myo-Inositol, stigmasterol, palmitic acid, stearic acid, caffeic-acid, D-pintol, ferulic acid, 4-hydroxybenzoicacid) were retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/) database in SDF file format, and then converted to the PDB form using the Open Babel server (O'Boyle et al., 2011). Afterwards, Open babel database was used to convert SDF files format (2D structure) to PDB format (3D structure). Open babel is freely available under an open-source license from http://openbabel.org, or https://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html. (Kumar, 2011).

The compound ID of the 3D structures of ligands was CID: 72 (3,4-Dihydroxybenzoic acid), CID: 135 (4-Hydroxybenzoic acid), CID: 7478 (4-Methoxybenzoic acid), CID: 222284 (beta-sitosterol), CID: 73145 (beta amyrin), CID: 1794427 (chlorogenic acid), CID: 12575 (Isovanillic acid), CID: 6549 (linalool), CID: 91704083 (lupeol trifluoroacetate), CID: 259846 (lupeol), CID: 892 (Myo-Inositol), CID: 5280794 (stigmasterol), CID: 985 (palmitic acid), CID: 5281 (stearic acid), CID: 689043 (caffeic acid), CID: 164619 (D-pintol), CID: 445858 (ferulic acid), and CID: 11005 (myristic acid).

During preparation, Gasteiger charge is added to the ligands using Autodock Tools. This software gives the information about the rotatable bonds for ligands. It also helps for the conversion of protein, ligands structure of the PDB format into the PDBQT (Protein Data Bank, Partial Charge (Q), & Atom Type (T)) format, which is essential for finding the binding affinity in Autodock tools (Jeong et al., 2019).

**2.3. Molecular docking**

Molecular docking was performed to predict the binding affinity between the ligands and the protein crystal structures using Autodock Tools. The phytochemical compounds were docked with each protein (AS160 and PTEN). For Autodock tools, empirical scoring functions help to calculate the binding affinity using Grid parameters, where blind docking is conducted in scanning the entire surface of protein targets as shown in Fig.4a. The size of the grid box (in Angstrome unit- Å-) was for PTEN (46 Å x 76 Å x 54 Å), and for AS160 (52 Å x, 62 Å x 54 Å). In each docking experiment, the target protein was kept rigid and the center of the grid was placed at the center of the mass of the original protein receptor, Fig.4b. Pymol software was used in finding the type of amino acids residues of the target protein that interact with the ligand; it is freely available under an open-source license from https://pymol.org/2/. Various types of

interactions occur at the binding interface between the protein and ligand, such as electrostatic, hydrophobic, and Van der Waals force interactions. (Monteiro et al., 2018, Zheng et al., 2019).

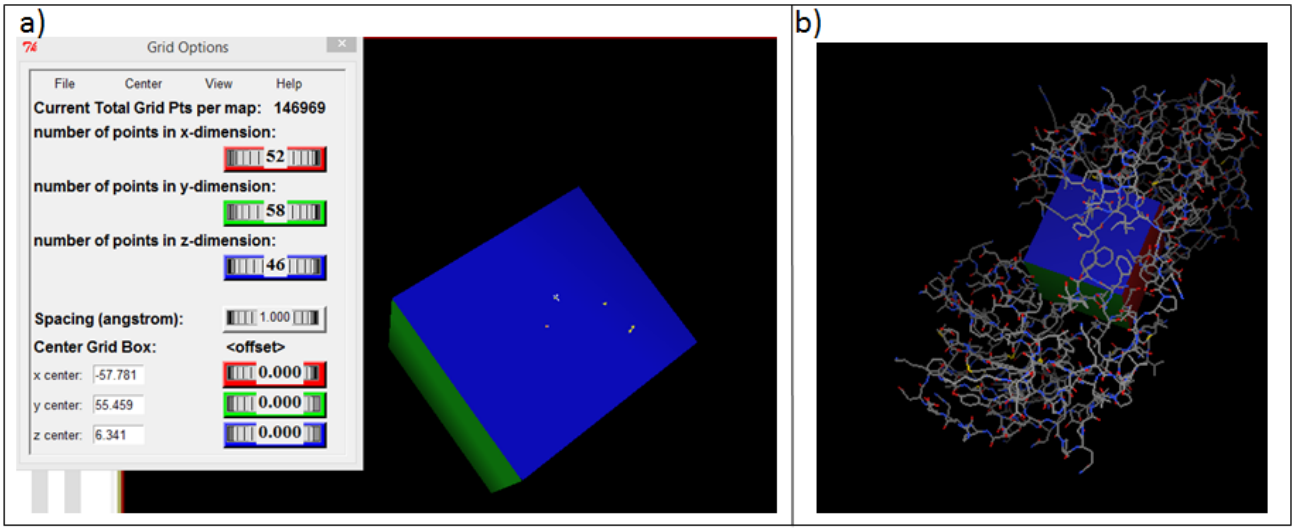



Figure (4): a- Scanning the entire surface of protein targets for the detection of possible binding sites.

b- Showed the center of the grid box which was placed at the center of the mass of the original protein receptor

**2.4. Ligand base ADME prediction**:

Swiss ADME is accessed at http://www.swissadme.chin, which is a free web browser that displays directly the submission page of SwissADME. Input parameters include physicochemistry, drug-likeness, pharmacokinetics and medicinal chemistry friendliness properties. Swiss-ADME is used to predict the (Absorption, Distribution, Metabolism and Excretion) properties for a chemical structure of the drugs. Drug molecules might fail during development because of several reasons. However found by the researchers one of the major reasons of failures is related with poor pharmacokinetic and absorption, distribution, metabolism and excretion (ADME) properties.

The SwissADME website was opened, files were imported from the external file option, and then files were converted into molecular sketcher based on ChemAxon's Marvin JS followed by

ADME calculation with default parameters. Chemical structure of compounds was downloaded from PubChem data bank (http://pubch em.ncbi.nlm.nih.gov/) in SDF (structure data format). By using Swiss ADME tool selected ligands will be screened based on the Lipinski rule of five (RO5) in order to find the drug likeness. The rules summarize the following physiochemical criteria's (Sharma et al., 2019)

- Molecular weight <500 Da
- Number of hydrogen bond acceptors ≤10
- Number of hydrogen bond donors ≤ 5
- Molecules should have logP ≤5
- Number of rotatable bonds <10

The bioavailability of drugs is given a low score if molecules will violate more than one of the aforementioned rules.

## 3. Results:

### 3.1. Insilico molecular docking Analysis

#### 3.1.1. Docking analysis of ligands against target AS160:

All seventeen ligands were tested for their binding affinities, inhibition constants, and the root mean square deviation (RMSD) values for the ligand structure upon docking, with no comparing with positive references, i.e., no *in vitro* experiment was performed for these phytochemicals or for a positive control as antidiabetic agent against PTEN or AS160. Furthermore, docking is performed blindly, where no prior information about the catalytic sites/ binding sites in a protein is present. Thus the whole protein surface was scanned for plausible binding site. The best docking is expected to have binding free energy lower than -5 kcal/mol and inhibition constant

less than 40 μM Five compounds showed strong binding with AS160 (Tables 1). This is based on their binding free energies in addition to the low values for the inhibition constant, Ki. These ligands are: beta-amyrin (Ki =18.06 μM), followed by stigmasterol (Ki = 28.48 μM), lupeol (Ki = 30.24μM), beta-sitosterol (Ki = 36.68μM) and lupeol-trifluoroacetate (Ki = 37.00μM). Few or no polar contacts were seen between these five ligands and the target protein's designated amino acid residues, while the majority of interactions were comprised of weak nonpolar contacts, see Fig. 5. However, for the other ligands, (3,4-Dihydroxybenzoic acid, 4-Methoxybenzoic acid, chlorogenic acid, Isovanillic acid, linalool, myo-Inositol, palmitic acid, stearic acid, caffeic acid, D-pintol, ferulic acid, and 4-hydroxybenzoicacid), the score of binding affinity was more than -5 kcal/mol. In terms of the polar and non-polar contacts (Table2), the situation is the opposite to the list of amino acids mentioned above and is classified having strong polar contacts and few non-polar contacts. Fig. 6. shows the most prominent and common amino acid residues binding to the target protein AS160; which were His-873, Lus-877 and Glu-1025; see Table 3.

Table 1: Best result of binding free energies, inhibition constants, and the RMSD values calculated .by AutoDock for the ligand binding to AS160 and PTEN

| NO. | Phytochemical | AS160 | | | PTEN | | |
|---|---|---|---|---|---|---|---|
| | | Autodock binding free energy (Kcal/mol) | Autodock inhibition constant (Ki) uM | RMSD (Å) | Autodock binding free energy (Kcal/mol) | Autodock inhibition constant (Ki) µM | RMSD (Å) |
| 1. | Beta-sitosterol | -6.05 | 36.68 | 66.170 | -8.55 | 536.40 nM | 98.465 |
| 2. | Beta-Amyrin | -6.47 | 18.06 | 93.828 | -6.75 | 11.31 | 95.018 |
| 3. | Lupeol-trifluoroacetate | -6.05 | 37.00 | 95.301 | -6.60 | 14.58 | 97.428 |
| 4. | lupeol | -6.17 | 30.24 | 93.823 | -6.45 | 18.67 | 95.525 |
| 5. | Stigmasterol | -6.20 | 28.48 | 61.894 | -7.88 | 1.67 | 98.025 |

Table 2: Binding free energies, inhibition constants, and the RMSD values calculated by AutoDock for the other ligands binding to AS160 and PTEN

| NO. | Phytochemical | AS160 | | | PTEN | | |
|---|---|---|---|---|---|---|---|
| | | Autodock binding free energy (Kcal/mol) | Autodock inhibition constant (Ki) uM | RMSD (Å) | Autodock binding free energy (Kcal/mol) | Autodock inhibition constant (Ki) uM | RMSD (Å) |
| 1 | 3,4-Dihydroxybenzoic-acid | -4.41 | 589.67 | 111.634 | -4.41 | 589.67 | 111.634 |
| 2 | 4-Hydroxybenzoicacid | -4.34 | 659.65 | 112.195 | -4.34 | 659.65 | 112.195 |
| 3 | 4-Methoxybenzoic-acid | -4.66 | 384.49 | 111.559 | -4.66 | 384.49 | 111.559 |
| 4 | Caffeic-acid | -4.15 | 912.34 | 97.421 | -4.15 | 912.34 | 97.421 |
| 5 | Chlorogenic-acid | -1.69 | 57.53 mM | 112.504 | -1.69 | 57.53 mM | 112.504 |
| 6 | D- pintol | -1.51 | 78.52 mM | 103.006 | -1.51 | 78.52 mM | 103.006 |
| 7 | Ferulic-acid | -4.41 | 590.05 | 88.522 | -4.41 | 590.05 | 88.522 |
| 8 | Isovanillic-acid | -4.30 | 708.66 | 111.460 | -4.30 | 708.66 | 111.460 |
| 9 | Linalool | -2.23 | 23.18 mM | 111.894 | -2.23 | 23.18 mM | 111.894 |
| 10 | Myo-Inositol | -2.22 | 23.45 mM | 84.885 | -2.22 | 23.45 mM | 84.885 |
| 11 | Palmitic-Acid | -2.98 | 6.59 mM | 93.566 | -2.98 | 6.59 mM | 93.566 |
| 12 | Stearic-acid | -0.87 | 230.46 mM | 100.108 | -0.87 | 230.46 mM | 100.108 |

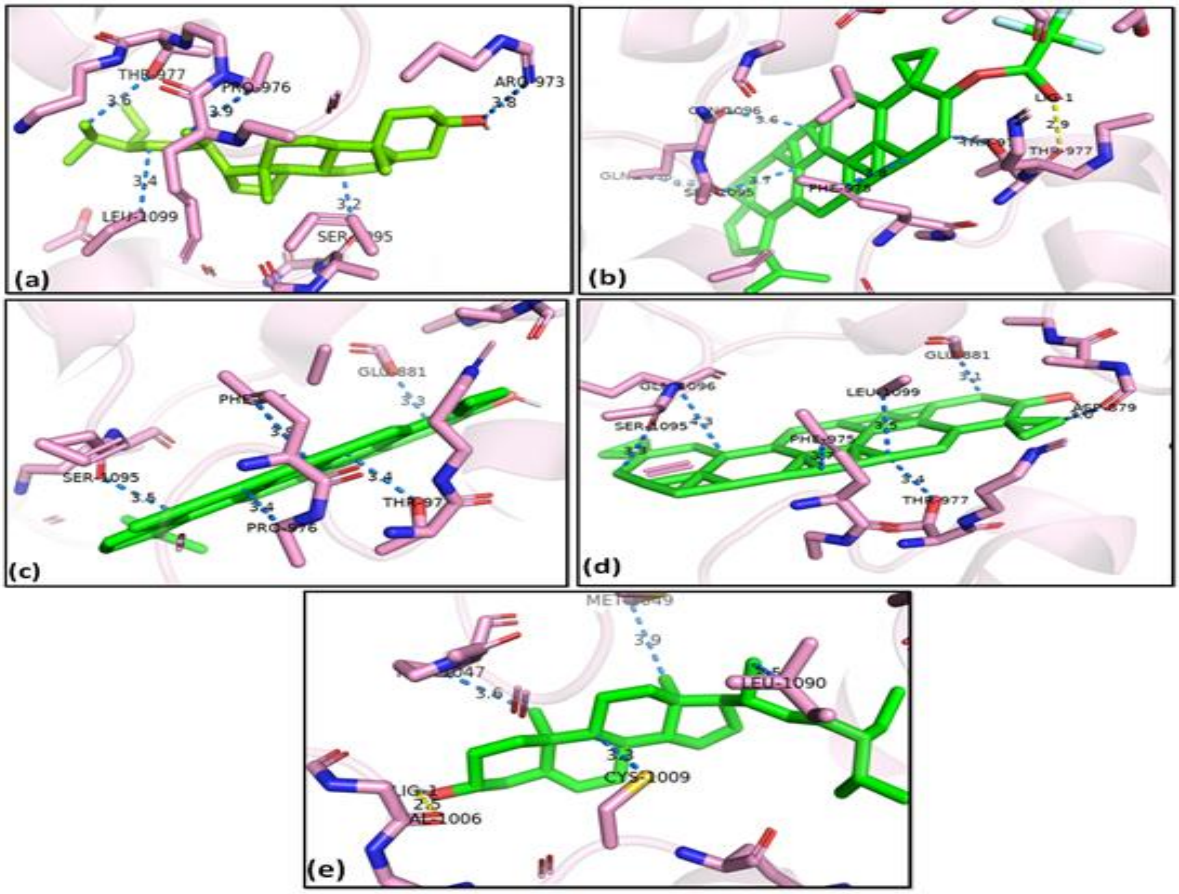



Figure 5: Binding interface between plausible inhibitors and the AS160 protein (pink): (a) Beta_sitosterol, (b) Lupeol,trifluoroacetate , (c) Lupeol, (d) Beta-Amyrin, and (e) Stigmasterol . All amino acids that are within 5 Å from the ligand are shown as sticks. The rest of the protein is shown in an 80% transparent cartoon model. Polar contacts are shown in yellow, whereas other possible contacts are in blue.

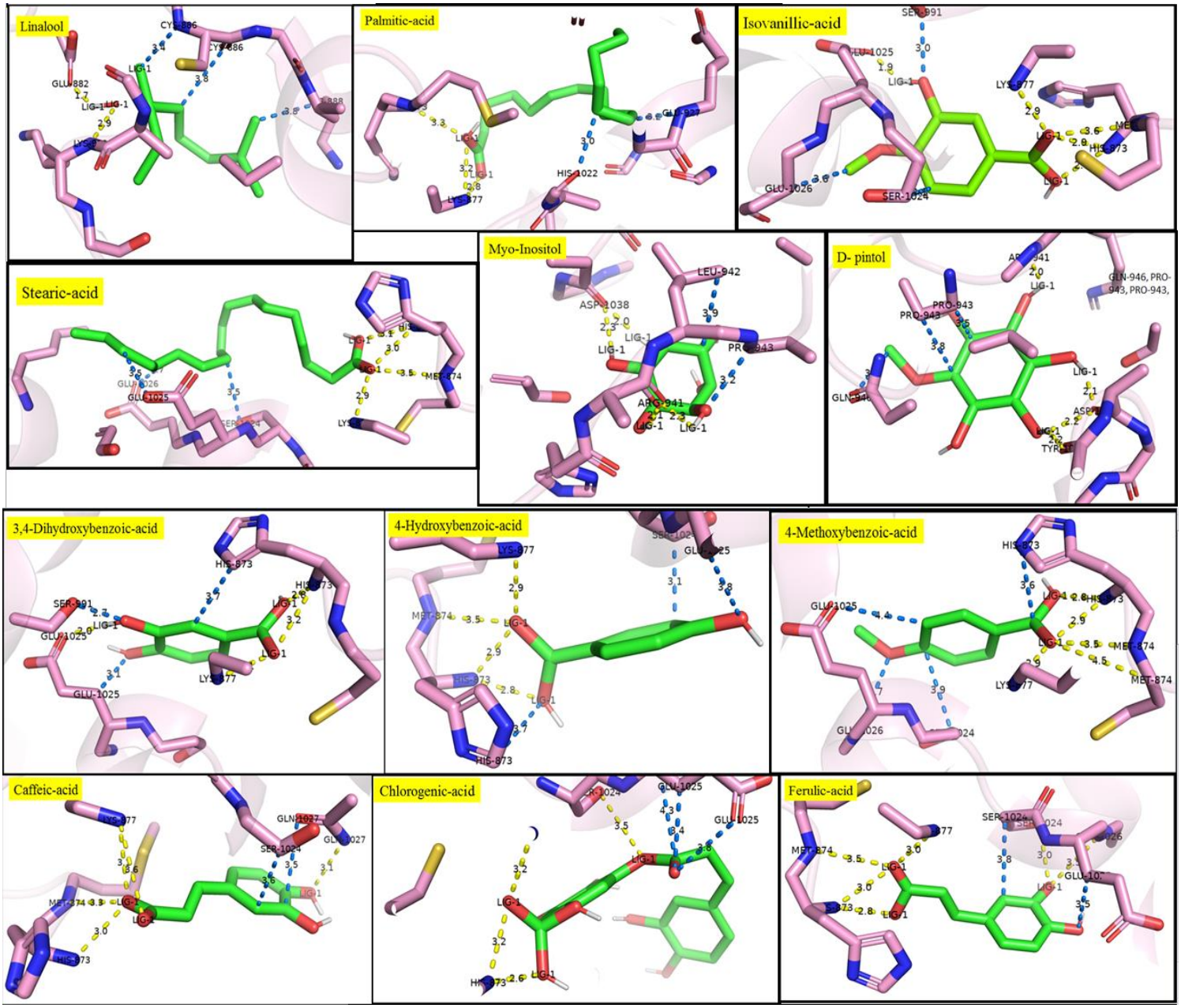



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Figure (6): Binding interface between plausible inhibitors ( green) and the *AS160* protein (pink) and the other ligands. All amino acids that are within 5 Å from the ligand are shown as sticks. The rest of the protein is shown in an 80% transparent cartoon model. Polar contacts are shown in yellow, whereas other possible contacts are in blue

Table 3: Docking analysis depicting the binding residues of AS160 protein target with ligands.

| # | Phytochemical | Number of polar contacts | **Polar contact binding residues** | Number of non-polar contacts | **Other intermolecular contact binding residues** |
|---|---|---|---|---|---|
| 1 | 3,4-Dihydroxybenzoic-acid | 4 | Glu-1025, HIS-873, HIS-873, LYS-877 | 3 | HIS-873, SER-991, GLU-1025 |
| 2 | 4-Hydroxybenzoicacid | 4 | LYS-877, MET-874, HIS-873, HIS-873 | 3 | SER-1024, GLU-1025, HIS-873 |
| 3 | 4-Methoxybenzoic-acid | 5 | MET-874, MET-874, LYS-877, HIS-873, HIS-873 | 4 | HIS-873, GLU-1025, GLU-1026, SER-1024 |
| 4 | Beta-sitosterol | 0 | – | 5 | Pro-976, Ser-1095, Leu-1099, Arg-973, Thr-977 |
| 5 | Beta-Amyrin | 0 | - | 7 | Glu-881, Leu-1099, Thr-977, Phe-975, Ser-1095, Gln-1096, Asp-879 |
| 6 | Caffeic-acid | 5 | LIS-877, MET-874, HIS-873, LYS-877, GLN-1027 | 2 | GLN-1027, SER-1024 |
| 7 | Chlorogenic-acid | 4 | SER-1024, HIS-873, HIS-873, LYS-877 | 3 | GLU-1025, GLU-1025, GLU-1026 |
| 8 | D-pintol | 4 | ASP-1038, ASP-1038, TYR-1037, ARG-941 | 3 | GLN-946, PRO-943, PRO-943 |
| 9 | Ferulic-acid | 6 | SER-1024, GLU-1026, LYS-877, HIS-873, HIS-873, MET-874 | 2 | SER-1024, GLU-1025 |
| 10 | Isovanillic-acid | 5 | GLU-1025, LYS-877, HIS-873, HIS-873, MET874 | 3 | SER-991, SER-1024, GLU-1026 |
| 11 | Linalool | 2 | LYS-922, GLU-882 | 3 | LYS-888, CYS-886, CYS-886 |
| 12 | Lupeol,trifluoroacetate | 1 | Thr-977, | 5 | Thr-977, Phe-975, Ser-1095, Gln-1096, Gln-1096 |
| 13 | lupeol | 0 | - | 5 | Glu-881, Thr-977, Pro-976, Ser-1095, Phe-975 |
| 14 | Myo-Inositol | 4 | ARG-941, ARG-941, ASP-1038, ASP-1038 | 2 | LEU-942, PRO-943 |
| 15 | Palmitic-Acid | 3 | LYS-877, LYS-877, HIS-873 | 2 | GLU-927, HIS-1022 |
| 16 | Stearic-acid | 4 | LYS-788, HIS-873, HIS-873, MET-874 | 3 | SER-1024, GLU-1026, GLU-1025 |
| 17 | Stigmasterol | 1 | Val-1006 | 4 | Leu-1090, Cys-1009, Pro-1047, Met-1049 |

### 3.1.2. Docking analysis of ligands against target PTEN:

The results for PTEN, presented in Table 1 correspond well with the results for AS160, where the same five ligands showed the highest binding affinities with the lowest Ki values. The phytochemicals are: beta-sitosterol (Ki =536.40 nM), followed by stigmasterol (Ki = 1.67 μM), beta-amyrin (Ki = 11.31 μM), lupeol-trifluoroacetate (Ki = 14.583 μM) and lupeol (Ki = 18.67 μM). Other potent inhibitors also showed low Ki with PTEN, including chlorogenic acid (Ki = 18.58 μM), but had a lower binding free energy compared to the five ligands mentioned above (-2.36 kcal/mol). Since there were few or no polar connections between the ligand and the specified amino acid residues in the target protein, weak non-polar contacts were responsible for the majority of interactions, Fig. 7. For the other ligands, however, the situation is exactly the opposite, where they had a lower binding free energy (Table 2), and most of the contacts were polar rather than non-polar, Fig. 8. The target PTEN was most frequently identified to bind to the amino acid residues Tyr-176, Arg-173, Lys-327, and Asn-323, Table 4.

### 3.2. Drug-likeness analysis of bioactive compound

Drug-likeness was analyzed to check whether bioactive compounds possess favorable ADME (absorption, distribution, metabolism, and excretion) properties. The good drug is expected to have these results; follow the Lipinski RO5, has good solubility, has no excretion problems, non-inhibitor of CYP- enzymes and specific in nature .A molecule is deemed to be not orally active if it violates two or more of the Lipinski Five Rules: molecular weight (MW) not exceeding 500 g/mol, hydrogen bond acceptors not exceeding 10, hydrogen bond donors not exceeding 5, and number of rotatable bonds not exceeding 10. Our calculations show that each ligand of the 17 compounds obeys the Lipinski's rule of five using SwissADME online web tool, Table 5, with some exceptions;

1- Chlorogenic-acid and myo inositol had a number of H-bond donor =6

2- The molecular weight of lupeol-trifluoroacetate is over 500, (MW=522.37g/mol).

3- Palmitic acid and stearic-acid had a number of rotatable bond more than 10 (14, 16) respectively.

Thus, all the compounds are orally active; as they do follow Lipinski RO5 (None of them has violation of two or more rule).

Other drug-likeness parameters of bioactive compounds are listed in Table 6 with their different parameters shown in SwissADME bioavailability radar in Fig. 9. The bioavailability score predicts the fraction of an orally administered compound that reaches systemic circulation. The bioavailability score ranges from 0 to 1, and a score closer to 1 indicates better bioavailability. Ligands that show best bioavailability (0.85 score) are; stearic acid, palmitic acid, ferulic acid, Isovanillic acid, 4-methoxybenzoic acid, and 4-hydroxybenzoic acid. On the other hand, lupeol and chlorogenic acid show the least bioavailability (0.17, 0.11) respectively. The remaining ligands (3,4-dihydroxybenzoic acid, beta-sitosterol, beta-amyrin , linalool, lupeol-trifluoroacetate, myo inositol, stigmasterol, caffeic acid, D-pintol) showed intermediary score (0.55 to 0.56). Compounds with drug-likeness should have a good aqueous solubility which is predicted by three methods: ESOL, (ALI) logS, and (SILICOS- IT) logS (Cui et al., 2020). 3,4-Dihydroxybenzoic-acid bioactive compound showed good solubility and no excretion problems, as there is no pharmacokinetics P-gp (permeability glycoprotein) interference. However, it is an inhibitor of CYP3A4 enzyme. The PAINS (Pan assay interference compounds) exhibited some interference of the 3,4-dihydroxybenzoic acid with catechol A compound, which indicates compound is not specific. 4-Hydroxybenzoic-acid, ferulic acid, Isovanillic acid, linalool and 4-

methoxybenzoic acid showed good solubility properties, are specific in nature (zero alerts for PAINS), had no excretion problems, and are non-inhibitors of CYP enzymes. Compounds such as beta-sitosterol, beta-amyrin and lupeol-trifluoroacetate showed poor solubility. Yet they have no excretion problems, are non-inhibitors of CYP enzymes, and the compounds are specific in nature. Caffeic acid is very soluble, has no excretion problems. It is a non-inhibitor of CYP enzymes, and it is specific in nature. Chlorogenic acid is nearly very soluble, and there is no excretion problems as there is no pharmacokinetics P-gp interference. Additionally, it is a non-CYP enzyme inhibitor. Nonetheless the PAINS exhibited some interference with Catechol A compound, which indicates compound is not specific. Myo inositol and D-pintol are highly soluble and non-inhibitors of CYP enzymes, and specific in nature (no interference with other compounds) but they have excretion problems. Lupeol showed no excretion problems, and it is a non-inhibitor of CYP enzymes. The phytochemical is specific in nature, but it is poorly soluble. Palmitic acid showed poor to moderate solubility with no excretion problems, and it is specific in nature, However, it is an inhibitor of CYP1A2 and CYP2C9 enzymes. Stearic acid showed poor solubility with no excretion problems and compound is specific in nature. Indeed, the molecule is an inhibitor of only CYP2A2 enzymes. The same properties of stearic acid apply to Stigmasterol, with the only difference for Stigmasterol being an inhibitor of only CYP2C9 enzyme.

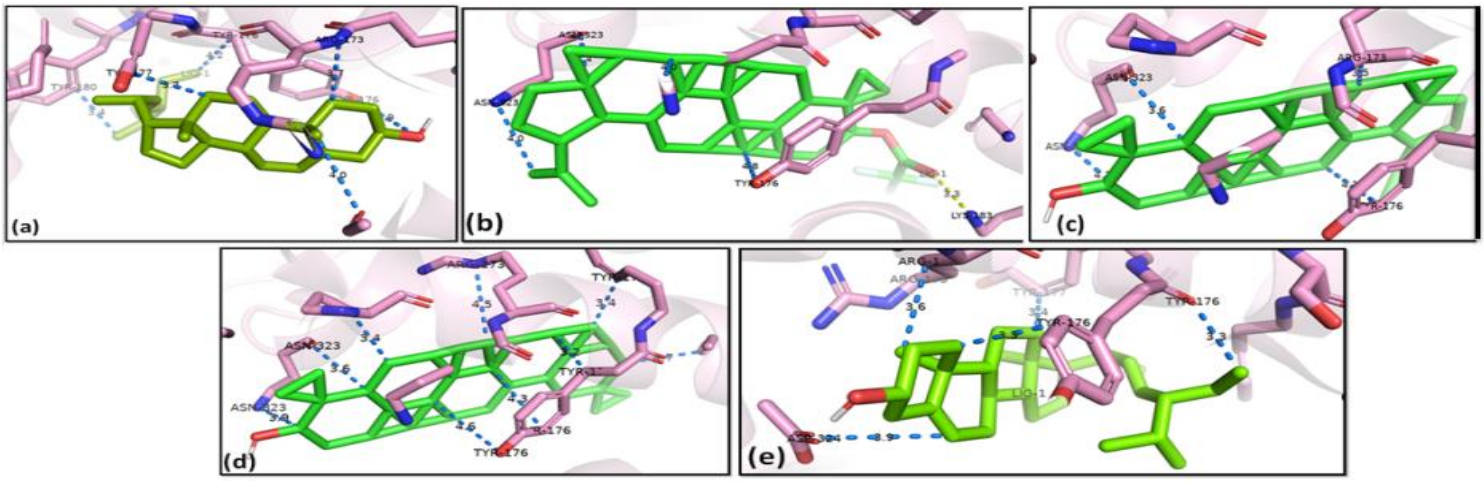


Figure 7: Binding interface between plausible inhibitors and the PTEN protein: (a) Beta-sitosterol, (b) Lupeol,trifluoroacetate , (c) Lupeol, (d) Beta-Amyrin, and (e) Stigmasterol . All amino acids that are within 5 Å from the ligand are shown as sticks. The rest of the protein is shown in an 80% transparent cartoon model. Polar contacts are shown in yellow, whereas other possible contacts are in blue.

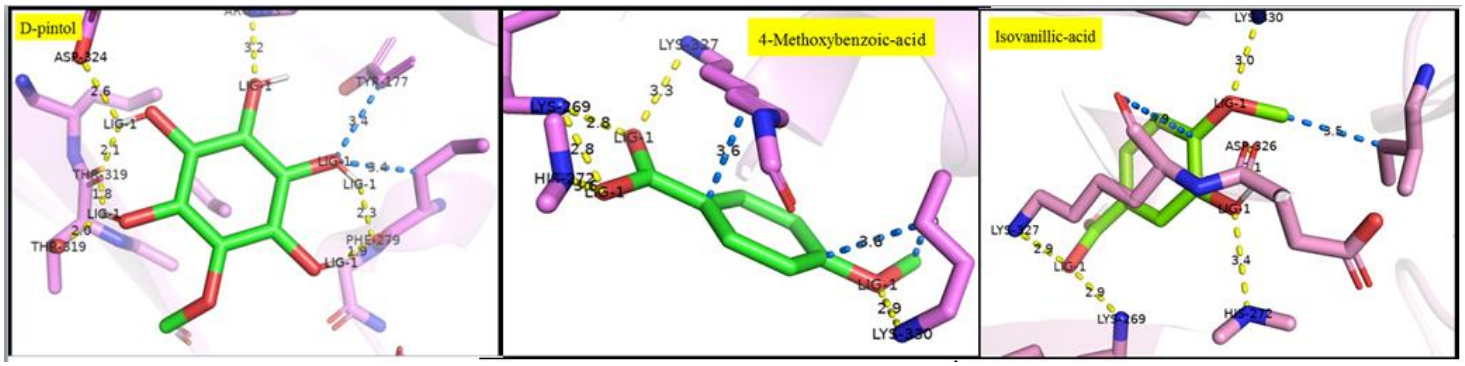


Figure 8: Binding interface between plausible inhibitors and the PTEN protein and the other ligands. All amino acids that are within 5 Å from the ligand are shown as sticks. The rest of the protein is shown in an 80% transparent cartoon model. Polar contacts are shown in yellow, whereas other possible contacts are in blue.

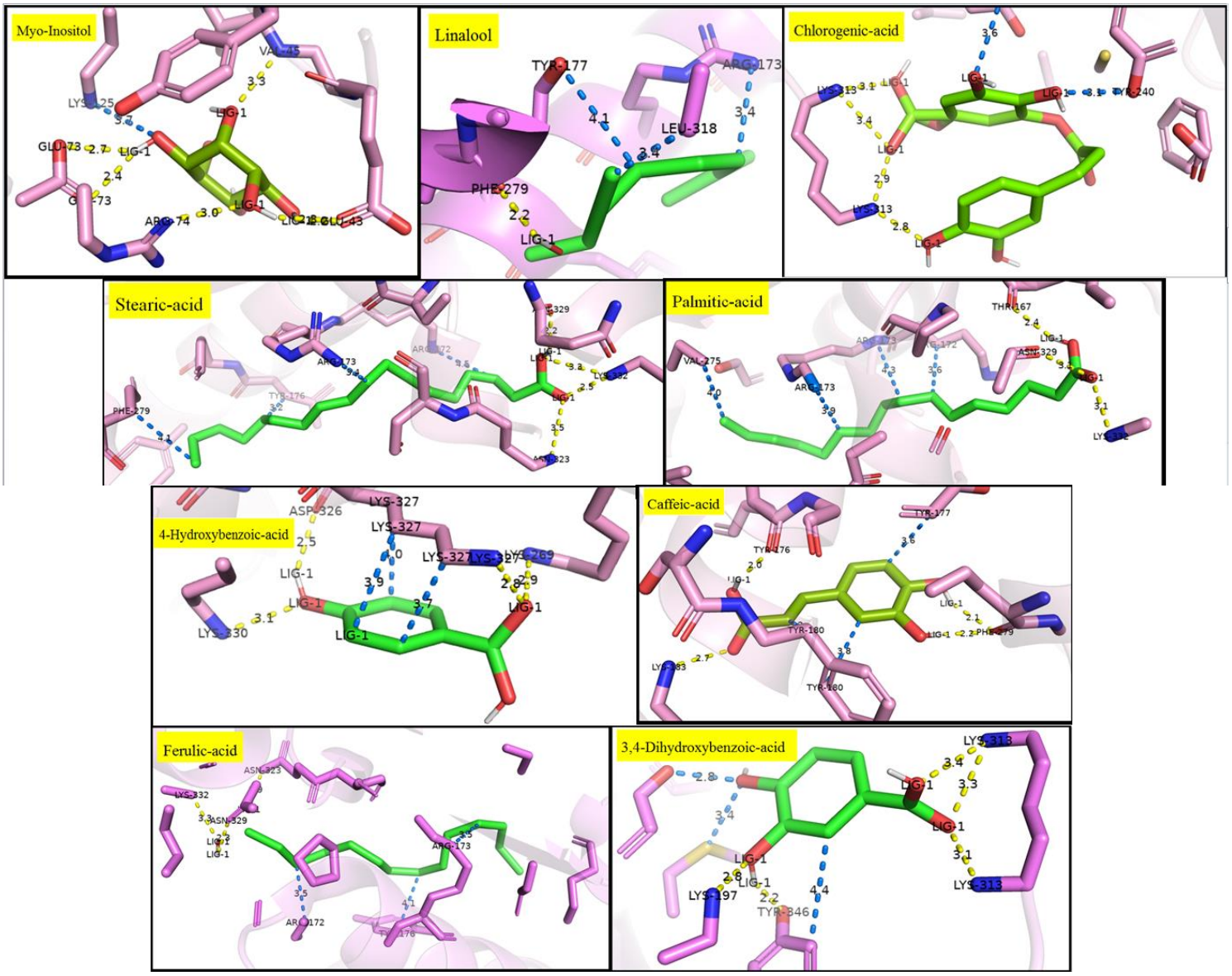


Figure 8 (continued): Binding interface between plausible inhibitors and the PTEN protein and the other ligands. All amino acids that are within 5 Å from the ligand are shown as sticks. The rest of the protein is shown in an 80% transparent cartoon model. Polar contacts are shown in yellow, whereas other possible contacts are in blue.

Table 4: Docking analysis depicting the binding residues of PTEN protein target with ligands.

| # | Phytochemical | Number of polar-contacts | **Polar contact binding residues** | Number of non-polar contacts | **Other intermolecular contact binding residues** |
|---|---|---|---|---|---|
| 1 | 3,4-Dihydroxybenzoic-acid | 5 | Lys-313, Lys-313, Lys-313, Lys197, Tyr346 | 3 | Tyr-346, Met-199, Tyr-240 |
| 2 | 4-Hydroxybenzoic-acid | 4 | Lys330, Asp-326, Lys-269, Lys-327 | 3 | Lys-327, Lys-327, Lys-327 |
| 3 | 4-Methoxybenzoic-acid | 3 | Lys-330, Lys-327, Lys-330 | 5 | Lys-327, Lys-269, His272, Lys-269, Lys330 |
| 4 | Beta-sitosterol | 0 | – | 6 | Tyr-176, Tyr-180, Tyr- 177, Arg-173, Asp-324, Tyr-176 |
| 5 | Beta-Amyrin | 0 | – | 9 | Tyr-176, Tyr-176, Tyr-176, Tyr-177, Tyr-180, Arg-173, Pro-169, Asn-323, Asn-323 |
| 6 | Caffeic-acid | 4 | Tyr-176, Lys-183, Phe-279, Phe-279 | 3 | Tyr-177, Tyr-180, Tyr-180 |
| 7 | Chlorogenic-acid | 4 | Lys-313, Lys313, Lys-313, Lys- 313 | 2 | Tyr-240, Lys-197 |
| 8 | D-pintol | 7 | Asp-324, Thr-319, Thr-319, Thr-319, Arg-173, Phe-279, Phe-279 | 1 | Tyr-177, Phe-279 |
| 9 | Ferulic-acid | 3 | Asn-329, Asn-323, Lys-332 | 3 | Arg-173, Tyr-176, Arg-172 |
| 10 | Isovanillic-acid | 5 | Lys-330, His-272, Lys-269, Lys-327, Asp-326 | 2 | Lys-327, Ile-168 |
| 11 | Linalool | 1 | Phe-279 | 3 | Arg-173, Leu-318, Tyr-177, |
| 12 | Lupeol-trifluoroacetate | 1 | Lys-183 | 4 | Asn-323, Asn-323, Tys-176, Arg-172 |
| 13 | lupeol | 0 | - | 4 | Tyr-176, Arg-173, Asn-323, Asn-323 |
| 14 | Myo-Inositol | 6 | Val-45, Glu- 73, Glu-73, Arg-74, Glu-43, Glu-43 | 1 | Lys-125 |
| 15 | Palmitic-acid | 3 | Lys-332, Asn-329, Thr-167 | 4 | Val- 275, Arg-173, Arg-173, Arg-172 |
| 16 | Stearic-acid | 4 | Lys-332, lys-332, Asn-323, Asn-329 | 4 | Arg-172, Arg-173, Tyr-176, Phe-279 |
| 17 | Stigmasterol | 0 | -- | 5 | Tys-176, Tyr-176, Asp-324, Arg-173, Tyr-177 |

Table 5: ADME analysis -Physicochemical properties (Lipinski rule of five of ligands)

| # | Phytochemical | Physicochemical properties (Lipinski rule of five) | | | | |
|---|---|---|---|---|---|---|
| | | Molecular weight (g/mol) | H-bond acceptor | H-bond donor | Log P | No of rotatable bond |
| 1 | 3,4-Dihydroxybenzoic-acid | 154.12 | 4 | 3 | 0.66 | 1 |
| 2 | 4-Hydroxybenzoic-acid | 138.12 | 3 | 2 | 0.85 | 1 |
| 3 | 4-Methoxybenzoic-acid | 152.15 | 3 | 1 | 1.56 | 2 |
| 4 | Beta-sitosterol | 414.71 | 1 | 1 | 4.79 | 6 |
| 5 | Beta-Amyrin | 426.72 | 1 | 1 | 4.63 | 0 |
| 6 | Caffeic-acid | 180.16 | 4 | 3 | 0.97 | 2 |
| 7 | Chlorogenic-acid | 354.31 | 9 | 6 | 0.96 | 5 |
| 8 | D-pintol | 194.18 | 6 | 5 | 0.36 | 1 |
| 9 | Ferulic-acid | 194.18 | 4 | 2 | 1.62 | 3 |
| 10 | Isovanillic-acid | 168.15 | 4 | 2 | 0.89 | 2 |
| 11 | Linalool | 154.25 | 1 | 1 | 2.70 | 4 |
| 12 | Lupeol-trifluoroacetate | 522.73 | 5 | 0 | 4.95 | 4 |
| 13 | Lupeol | 426.72 | 1 | 1 | 4.68 | 1 |
| 14 | Myo-Inositol | 180.16 | 6 | 6 | 0.31 | 0 |
| 15 | Palmitic-acid | 256.42 | 2 | 1 | 3.85 | 14 |
| 16 | Stearic-acid | 248.48 | 2 | 1 | 4.30 | 16 |
| 17 | Stigmasterol | 412.69 | 1 | 1 | 5.01 | 5 |

Table 6: Drug-likeness analysis of bioactive compounds

| # | Phytochemical | Bioavailability score | Solubility | | | Pharmacokinetics | |
|---|---|---|---|---|---|---|---|
| | | | Log S (ESOL) | Log S (Ali) | Log S (SILICOS-IT) | GI absorption | CYP enzymes inhibitors |
| 1 | 3,4-Dihydroxybenzoic-acid | 0.56 | Very soluble | soluble | soluble | high | Only CYP3A4 |
| 2 | 4-Hydroxybenzoic-acid | 0.85 | soluble | soluble | soluble | high | No |
| 3 | 4-Methoxybenzoic-acid | 0.85 | soluble | soluble | soluble | high | No |
| 4 | Beta-sitosterol | 0.55 | Poorly soluble | Poorly soluble | Poorly soluble | low | No |
| 5 | Beta-Amyrin | 0.55 | Poorly soluble | Poorly soluble | Poorly soluble | low | No |
| 6 | Caffeic-acid | 0.56 | Very soluble | soluble | soluble | high | No |
| 7 | Chlorogenic-acid | 0.11 | Very soluble | soluble | soluble | low | No |
| 8 | D-pintol | 0.55 | highly soluble | highly soluble | soluble | low | No |
| 9 | Ferulic-acid | 0.85 | soluble | soluble | soluble | high | No |
| 10 | Isovanillic-acid | 0.85 | soluble | soluble | soluble | high | No |
| 11 | Linalool | 0.55 | soluble | soluble | soluble | high | No |
| 12 | Lupeol-trifluoroacetate | 0.55 | Poorly soluble | insoluble | Poorly soluble | low | No |
| 13 | lupeol | 0.17 | insoluble | insoluble | Poorly soluble | low | No |
| 14 | Myo-Inositol | 0.55 | highly soluble | highly soluble | soluble | low | No |
| 15 | Palmitic-acid | 0.85 | Moderately soluble | Poorly soluble | Moderately soluble | high | CYP1A2, CYP2C9 |
| 16 | Stearic-acid | 0.85 | moderately soluble | Poorly soluble | Poorly soluble | high | Only CYP2A2 |
| 17 | Stigmasterol | 0.55 | Poorly soluble | Poorly soluble | moderately soluble | low | Only CYP2C9 |

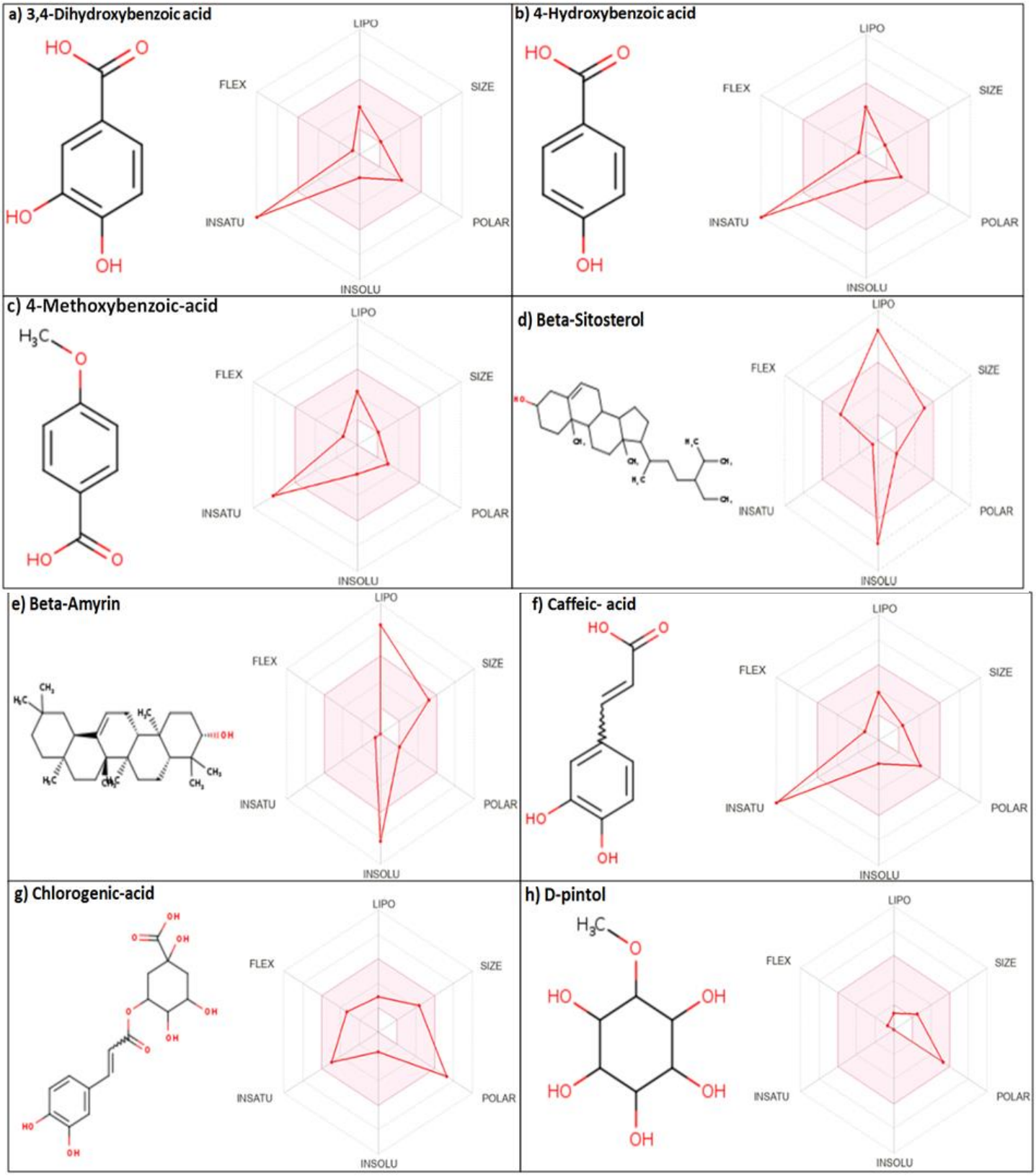


Figure 9: The Bioavailability Radar of different bioactive drug-likeness molecules where the pink areas represent each property (lipophilicity, molecular weight, solubility, and flexibility), with their 2D structures.

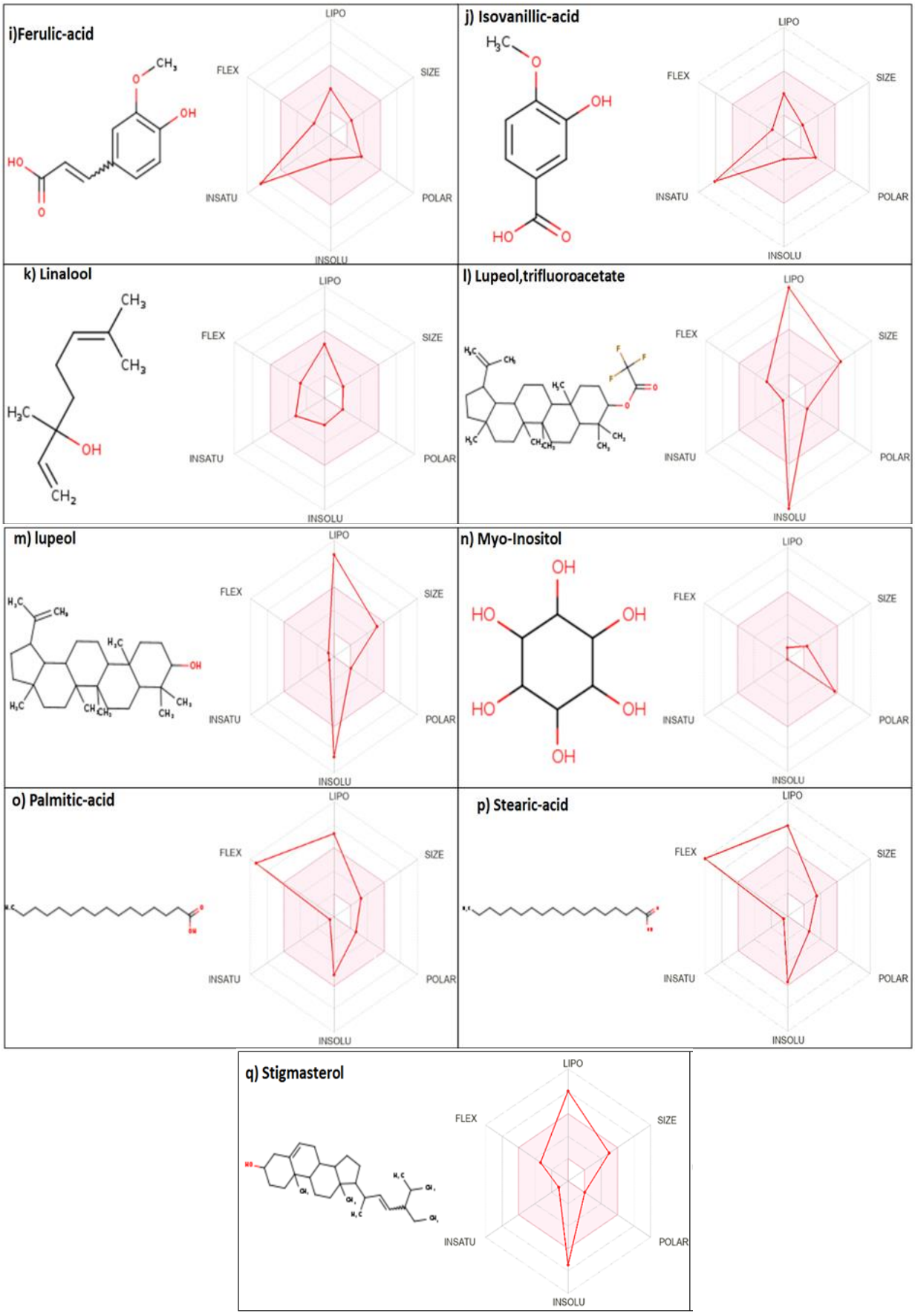


Figure 9 (continued): The Bioavailability Radar of different bioactive drug-likeness molecules where the pink areas represent each property (lipophilicity, molecular weight, solubility, and flexibility), with their 2D structures.

## 5. Discussion

*In silico* drug discovery is a handy and well-known technique that makes it possible to find novel compounds with therapeutic potentials. The method is based on information about protein structure. It is a structure-based drug design method that simulates the molecular interaction and predicts the binding mechanism and affinity between receptors and ligands. These processes, however, take a lot of time and money to complete. By analyzing enormous databases of molecules in virtual screening campaigns, computer-aided approaches have arisen as a tool to reduce the time and financial expenses of experimental trials. These techniques make it possible to select from big databases chemicals that may be active against a protein target. For a given ligand and receptor, AutoDockTools offers an interactive approach for defining the torsional tree and specification of ligand and receptor flexibility. The steps in this procedure have all been automated to enable automatic assignment for use in batch operations like virtual screening. Ligand flexibility is determined over a number of steps. As the fixed point during coordinate transformation in the docking simulation, a root atom is first selected. In order to determine the optimal atom, the atoms in each branch are counted, and the root atom that reduces the size of the greatest branch is selected. The user could occasionally want to reduce the ligand's flexibility in some case (Morris et al., 2009). The ability to undertake docking studies using Autodock or Autodock/Vina, combined with the ability to use Python scripts to automate image creation (Seeliger & de Groot, 2010) facilitates our study. All the ligands were examined for their binding affinities, inhibition constants, and root mean square deviation (RMSD) values in order to determine the mechanism by which each ligand would inhibit the two proteins Out of 17 compounds, 5 show high binding affinity with both target proteins; PTEN and AS160 and seem

to be the same but with different binding affinity. These 5 compounds also have lowest inhibition constant, and they are: beta-sitosterol, stigmasterol, beta-amyrin, lupeol-trifluoroacetate, and Lupeol. The inhibition constants for both proteins were also at their lowest values for the same five phytochemicals. However, because it is rigid docking (only the ligand is flexible), the RMSD measurements did not provide sensible value, hence these values were disregarded. Both polar and non-polar groups interact during docking, which is crucial for the stability of protein-ligand interactions. Most docking programs eliminate the water solvent because it frequently forms hydrogen bonds with molecules, either as a donor or acceptor. However, when two molecules are close to one another, the association between the two molecules has a negative value, and repulsive forces result (Sahin et al., 2006). The results highlight the fact that this high binding affinity was primarily caused by nonpolar interactions; furthermore, polarity did not contribute to a more stable binding interface and the binding free energy as the hydrophobic contacts did (Ishibashi et al., 2020).

In contrast, for both PTEN and AS160, these five compounds displayed more nonpolar contacts than the other ligands. Van der Waals interaction may be considered weak, but they play a vital role in structure and biomolecules interaction. Hydrogen bonds are also weak non-covalent bonds, which induce thermal fluctuation in energies and tend to form or break rapidly, causing conformational changes during binding. The drug-likeness parameters described are the main criteria in primary drug development. The 17 compounds studied follow Lipinski's rule of five, with some exceptions but none of them has violation of two or more rule (see results section #3.2 and table 5). Thus all of them are orally active . The best bioavailability is found in stearic acid, palmitic acid, ferulic acid, Isovanillic acid, 4-methoxybenzoic-acid, and 4-hydroxybenzoic-acid. Lupeol and chlorogenic-acid have the least bioavailability (0.17, 0.11). The remaining ligands

have intermediate scores (0.55 to 0.56). 3, 4-dihydroxybenzoic-acid bioactive compound has good solubility and no excretion problems. On the other hand, 4-hydroxybenzoic-acid, ferulic-acid, isovanillic-acid, linalool, and 4-methoxybenzoic-acid show good solubility properties and are non-inhibitors of CYP enzymes. Myo-Inositol and D-pintol are highly soluble and non-inhibitors of CYP enzymes but have excretion problems, while palmitic-acid is specific but an inhibitor of CYP1A2 and CYP2C9 enzymes. Although these five ligands (stigmasterol, beta-amyrin, lupeol, beta-sitosterol, and lupeol-trifluoroacetate) have the highest binding affinities and obey the Lipinski RO5 properties, all of them have no to poor solubility in nature which makes them unstable in GI absorption. In addition, stigmasterol is an inhibitor of CYP2C9 enzyme. Although ferulic acid, Isovanillic acid have moderate binding affinities, they may have more potential as drug-likeness against diabetes, because they abide by ADME characteristics and drug likeliness parameters.

## 4. Conclusion

Molecular docking analysis is a structure-based design of drugs. Despite protein-ligand docking's success in numerous virtual screening initiatives; there are a number of limitations. For instance, the protein target's flexibility is typically only partially considered. Some strategies employ merging and shrinking techniques as well as numerous reference target structures to solve this problem. Another drawback is the requirement for extensive prior biological system information, such as selecting the proper active site, determining the protonation status of an organism's amino acids, or specifying the kind of activity being sought after in ligands. Additionally, the effectiveness of the scoring procedure is crucial to the enrichment of the hit-list with real ligands.

One significant drawback of AutoDockTools is their dependence on data for the root mean square deviation (RMSD). For exact findings, it is necessary to allow both the ligand and the protein to be flexible (Morris et al., 2009). However, adding flexibility has several drawbacks. First, it requires more computation to calculate the receptor's (target protein's) energy because flexible regions must be assessed using a full pairwise energy evaluation. Second, because the conformational space is larger, there is a higher likelihood of false positives. The values of the inhibition constants and the Autodock binding free energy (kcal/mol) may be sufficient to assess the results, though more research is required. Plants have historically been the primary source of key natural substances used in medicine. This study represents molecular docking of 17 conpounds; (3,4-dihydroxybenzoic acid, 4-methoxybenzoic acid, beta-sitosterol, beta amyrin , chlorogenic acid, Isovanillic acid, linalool, lupeol trifluoroacetate, lupeol, myo-inositol, stigmasterol, palmitic acid, stearic acid, Caffeic acid, D-pintol, ferulic acid, 4-hydroxybenzoicacid) from the medical plants; *Gundelia tournefortii* and *Ocimum basilicum aginst* PTEN and AS160 main proteins in the insulin signaling pathway, The action mechanisms of the seventeen compounds, some of which have not yet been found, have not been explored or mentioned as prospective anti-diabetic drugs (Kadan et al., 2021). Additional to drug-likeness study. Stigmasterol, beta-amyrin, lupeol, beta-sitosterol, and lupeol-trifluoroacetate exhibit high binding affinities with good Autodock inhibition constant (Ki) against both PTEN and AS160. They have very high drug-likeness properties except that they are nearly insoluble in nature which makes them unstable to be absorbed in gastrointestinal tract. Additionally, stigmasterol inhibits the activity of the CYP2C9 enzyme. Regardless of having a moderate binding affinity, ferulic acid and isovanillic acid may be more effective drugs against diabetes since they conform to ADME features and drug likeliness factors. Another limitation of this study is that the docking

was performed with no reference to a positive control. Indeed, no crystal structure of the target proteins was found with boned inhibitors, which leads to a blind docking protocol. Herein, the whole protein surface was scanned by us for plausible binding sites. Furthermore, no *in vitro* experiment was performed for these phytochemicals to scan their pharmacokinetics properties in binding to PTEN or AS160.

For the purpose of validating the results of the current investigations, additional work must be done through drug identification using enzyme assays. To fully understand the mechanism of action and cytotoxicity studies on the aforementioned powerful bioactive chemicals, an *in vitro* and *in vivo* investigation must be carried out which will insure and support our results. Looking forward to the future, one of the goals of the project is also make docking between these seventeen phytochemicals with other targets protein that are found in the insulin signaling cascade.

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## الملخص

### رؤيا سيليكونية- محوسبة- لمواد كيميائية ذات مصدر طبيعي تؤثر على بروتينات رئيسية في مسار الانسولين: AS160 وPTEN

الأنسولين هو الهرمون الذي ينظم مستويات السكر في الدم. وهو يرتبط في مستقبلاته في الكبد، والعضلات، والخلايا الدهنية. وهذا يؤدي إلى امتصاص الجلوكوز و تحويله على شكل جليكوجين أو الدهونز وهي عملية تنطوي على بروتينات وإنزيمات متعددة، بما في ذلك PTEN و AS160 و هما بروتينان تنظيميان في مسار الانسولين، حيث انهما يثبطان سلسلة عمل البروتينات الأخرى الموجودة في سلسلة مسار تنشيط البروتينات استجابة الإنسولين. ويمكن أن تؤدي مقاومة الأنسولين إلى ارتفاع مستويات الجلوكوز في الدم، مما يسبب مرض السكري. وقد رصد في أبحاث سابقة عن مركبات طبيعية مستخلصة من أنواع مختلفة من النباتات مثل الإينوسيتول، والغلوكسيرانوز، وحامض ألفا-لينولينيك، بوصفها عوامل مضادة للسكر. وتهدف هذه الدراسة الى فحص ترابط كل من البروتينات التي تم ذكرها سابقا مع المواد الكيميائية وحساب الطاقة لهذا الترابط باستخدام البرامج المحوسبة ورصد المركبات الكيميائية الطبيعية كمثبطات لهذه البروتينات لمعالجة السكري، ويتحقق هذا الهدف باختبار التفاعل بين مختلف هذه المواد الكيميائة ذات المصدر الطبيعي- النباتات و الأعشاب الطبية من الريحان و العكوب مع AS160 وPTEN باستخدام أدوات AutoDock. ونهدف إلى العثور على الأحماض الأمينية لكل من AS16 0وPTEN التي تتفاعل مع هذه المركبات الكيميائية النباتية المختبرة باستخدام برمجيات Pymol وقد تم أيضاً في هذه الدراسة التعرف على خصائص االمركبات الشبيهة بالأدوية وخاصيتها (الامتصاص والتوزيع والاستقلاب الى خروجه من الجسم )

باستخدام أداة الويب SwissADME حيث تم فحص جميع االمواد الكيميائية المختارة استناداً إلى قاعدة ليبنسكي البالغة خمسة قواعد من أجل العثور على تشابه المواد الكيميائية وغيرها في خصائص العقاقير. وقد أظهرت عدة مركبات وجود صلة ارتباطية عالية مع البروتينات المستهدفة مثل beta-sitosterol, beta-amyrin, lupeol-trifluoroacetate, lupeol and Stigmasterol. وبالنسبة لتحليل swissADME ,حللت الدراسة 17 مركباً، وفقاً لقاعدة ليبنسكي، مع أفضل النتائج الموجود في stearic-acid, palmitic-acid, ferulic-acid, Isovanillic-acid, 4-methoxybenzoic-acid, and 4-hydroxybenzoic-acid. التي تظهر قدرتهم العالية على الذوبان ولكن غير متخصصة في هدفها. بينما Beta-sitosterol, beta-Amyrin, and lupeol-trifluoroacetate تُظهر قدرة ضعيفة على الذوبان ولكن متخصصة في هدفها.

هذه النتائج تشير إلى أن بعض المركبات الكيميائية يمكن أن تكون عقاقير مضادة للداء السكري مستقبلا بعد ان يتم اختبارها في النظم المختبرية والحيوية.