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Theme

Study of the inhibitory effect of some cypress metabolites on alpha amylase

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Abstract

Diabetes mellitus (DM) is the inability of the body to manage glucose levels, which leads to severe complications. The control of postprandial hyperglycemia is an important target in the treatment of DM. The synthetic anti-DM drugs have inevitable side effects; therefore, the investigation of natural compounds with little or no side effects as α-amylase inhibitors is of notable concern. In the present study, we predict the ability of *Cupressus sempervirens* L. compounds such as Cupressuflavone (Mol1), Robustaflavone (Mol2), Apigenin-4'-glucoside (Mol3), Sugiol (Mol4), Podocarpusflavone A (Mol5), apigenin-7-O-beta-D-glucopyranoside (Mol6), Hinokiflavone (Mol7), Kaempferol-3-O-rhamnoside (Mol8) as human pancreatic α-amylase inhibitors by *in silico* study. The molecular docking study was performed using AutoDock vina and prediction of its bioavailability and biological activity with the ADMET and PASS servers. The visualization of the molecular interactions was established in the Discovery studio software. The molecular docking results of Podocarpusflavone A and Robustaflavone revealed the best binding energies with values of -11.6 kcal/mol, -11.4 kcal/mol, and they had a good ADMET profile. These compounds might become drug candidates for drug discovery against type 2 diabetes.

Keywords: α-amylase, diabetes, *Cupressus sempervirens* L., molecular docking, ADMET, PASS.

Résumé

Le diabète sucré est une maladie liée au dysfonctionnement des mécanismes biologiques de régulation de la glycémie, ce qui peut entraîner de graves complications. Son traitement se base sur la gestion de l'hyperglycémie postprandiale par des médicaments synthétiques contre le diabète qui ont des effets secondaires indésirables et aigües. La recherche de composés naturels sans effets secondaires comme des inhibiteurs de l'α-amylase est d'une grande importance. Cette étude a pour but de déterminer la capacité des composés de *Cupressus sempervirens* L. tels que la Cupressuflavone (Moll), la Robustaflavone (Mo12), l'Apigénine-4'-glucoside (Mol3), le Sugiol (Mo14), le Podocarpusflavone A (Mol5), l'Apigénine-7-0-beta-D-glucopyranoside (Mol6), le Hinokiflavone (Mol7), le Kaempférol-3-0 rhamnoside (Mol8) comme des inhibiteurs de l'α-amylase pancréatique humaine dans le cadre d'une étude *in silico*. L'étude d'amarrage moléculaire a été réalisée à l'aide du logiciel AutoDock vina et la prédiction de la biodisponibilité et de l'activité biologique à l'aide des serveurs en ligne ADMET et PASS. La visualisation des interactions moléculaires a été établie en utilisant le logiciel Discovery studio visualizer. Les résultats de l'amarrage moléculaire de la podocarpusflavone A et de la Robustaflavone ont révélé être les meilleurs par rapport à leur affinité avec l'enzyme avec des énergies de -11,6 kcal/mol et -11,4 kcal/mol, et présentaient un bon profil ADMET. Ces composés ont un potentiel de devenir des candidats pour la découverte des médicaments pour le traitement contre le diabète type 2.

Mots-clés : α-amylase, diabète, *Cupressus sempervirens* L., amarrage moléculaire, ADMET, PASS.

ملخص

داء السكري، هو مرض أيضي يتصف بارتفاع نسبة السكر في الدم يحدث عندما يعجز البنكرياس عن إنتاج الإنسولين بكمية كافية، أو عندما يعجز الجسم عن الاستخدام الفعال للإنسولين الذي ينتجه. تمحورت دراستنا حول البحث عن مثبطات جديدة لإنزيم ألفا أميلاز البشري البنكرياسي لعلاج داء السكري الذي يعد أزمة صحية عمومية هامة. يهدف هذا العمل الى تقييم التأثير المثبط لسبع مركبات فينولية ومركب تيربيني ثنائي من نبات السرو دائم الخضرة في السيليكون على ألفا أميلاز بواسطة الالتحام الجزيئي بمساعدة البرامج: اتو دوك فينا، اتو دوك تولس وديسكو فري ستوديو فيزيا ليزور، متبوعة بحساب خصائص حركية الدواء: الامتصاص، التوزيع، عملية الأيض، الإطراح والسمية (أ د م ت) لهذه المثبطات باستخدام موقع سويس أدمت وأدمت لاب عبر الإنترنت. أفضل المثبطات المصنفة اختيرت وفقا لقيمة الطاقة الأقل الصادرة عن أدف وإعداد أدمت، كنتيجة، المركب روبيستافلافون (2) والمركب بودوكاربيسفلافون (5) صنفت على أنها أفضل مثبطات بناء على قيمة طاقة ربطها المسجلة ب 11.4- و11.6- كيلو كالوري/ مول ومعدل تكرارهما مع أفضل إعداد أدمت حيث قدمت كمثبطات محتملة جديدة، صنفت المثبطات المتبقية على أنها جيدة مقارنة ب المركب (5). هذه النتائج يمكن أن تكون هامة لاكتشاف أدوية جديدة لكن الدراسات الأخرى المخبرية والحيوية ضرورية لتأكيد فعاليتها.

كلمات مفتاحية: ألفا أميلاز، داء السكري، السرو دائم الخضرة، ماحتلاالجزيئي، امتصاص، توزيع، سمية وطرح.

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A-B

ADV: AutoDock Vina
ADT: AutoDock Tools
ADMET: Absorption, Distribution, Metabolism, Elimination and Toxicity.
Å: Angstrom
AD: *Anno Domini*
ASP: Aspartic acid
ALA: Alanine
ARG: Arginine
BC: before Christ
BBB: Blood brain barrier

C-E

Caco-2: Human Colon Carcinoma
CGM: Continuous glucose monitoring
CNAS: Caisse Nationale des Assurances Sociales.
CS: *Cupressus sempervirens* L.
CSII: Continuous subcutaneous insulin infusions
CYP: Cytochrome P450
DSV: Discovery Studio Visualizer

F-H

HERG: Human Ether Related Gene
HIA: Human Intestinal Absorption
HPSCs: Human pluripotent stem cells
HIS: Histidine
GLU: Glutamic acid
GLN: Glutamine
GLY: Glycine

I-K

iPSC: induced pluripotent stem cell
ILE: Isoleucine

L-O

MDI : Multiple Daily injection
LEU: Leucine
LYS : Lysine
TRP : Tryptophan

P-S

PASS: Prediction of Activity Spectra for Substances
PDB: Protein data bank
PDBQT: Protein Data Bank, Partial Charge (Q), Atom Type (T)
RR: repetition ratio
SAR: Structure Activity Relationship

T-X

T1DM: Type one Diabetes mellitus
T2DM: Type two Diabetes mellitus
THR: Threonine
TYR: Tyrosine

Table of content

INTRODUCTION

A disease characterized by abundant discharge of urine traces its origins to ancient times back to Egyptian manuscripts [**1**]. In his Samhita manuscript dating back to around 1500 B.C., the renowned Indian surgeon Sushruta made a significant observation and recognition of diabetes. He referred to this condition as "*madhumeha*" meaning "honey-like urine". Sushruta's description not only highlighted the sweet taste of the urine but also emphasized its sticky texture and its ability to attract ants [**2**]. The term "diabetes" is derived from the Greek word "*diabetes*" in the 2nd century AD [**3**]. It literally translates to "passing through" or "to go through" reflecting the early perception of a disease that involved excessive fluid loss [**4**].

The term "diabetes mellitus" was introduced by Thomas Willis, a British scientist, to establish a clinical distinction between this disease and diabetes insipidus. He also provided a clear description of its symptoms [**5**].

The discovery of insulin in 1922 represented a significant breakthrough in the field of medicine and revolutionized the treatment of patients with diabetes [**6**].

According to the International Diabetes Federation, 6.7 million deaths were caused by diabetes mellitus in 2021. With 537 million adults living with it, this number is predicted to rise to 643 million by 2030 and 783 million by 2045 [**7**]. The most common type of diabetes is Type 2 diabetes mellitus (T2DM), accounting for over 95% of all diabetes cases globally [**8**].

One of the therapeutic approaches of managing hyperglycemia in Type 2 Diabetes Mellitus is inhibiting the digestion of carbohydrates consumed in a meal [**9**].

Slowing down the digestion of carbohydrates and delaying the absorption of glucose, which leads to a decrease in blood sugar levels, can be achieved by inhibiting the pancreatic alpha-amylase enzyme. There are several drugs available on the market that act as inhibitors for alpha-amylase, including acarbose, miglitol, and voglibose [**10**]. These medications have been proven to

effectively target alpha-amylase. However, it's important to note that along with their benefits, these drugs are associated with certain side effects such as abdominal discomfort, diarrhea, and flatulence [**11**].

Products derived from plants are extensively used in drug development programs, principally for their availability, low cost and to avoid the side effect of synthetic drugs. Currently, medicinal plants are recommended for treating various diseases, including diabetes.

Among medicinal plants, *Cupressus semprevirens* L., a tall evergreen tree native to the Mediterranean region, is considered to be a traditional medicinal plant. It is used to treat many diseases, including diabetes, inflammation, stomach pains and cough [**12**].

The aim of this study is to investigate the potential of previously unstudied compounds derived from *Cupressus sempervirens* L. as inhibitors of human pancreatic α-amylase via docking analysis and ADMET prediction to evaluate their potential to become clinical antidiabetic drugs.

The manuscript is organized into three sections arranged as follows:

- The first part is bibliographical review where we presented an overview of: diabetes mellitus, description of *Cupressus sempervirens* L. and α-amylase.
- The second part is experimental, discussing the different used methods in this in silico study.
- The last part presents the obtained results and their discussion, followed by a conclusion and perspectives.

LITERATURE

REVIEW

1. Diabetes mellitus

Diabetes mellitus (DM) is a chronic and severe metabolic condition that greatly affects the quality of life and overall health of individuals. Characterized by high blood sugar levels, also known as hyperglycemia, due to inadequate insulin secretion, its insufficient action, or a combination of both [**13**]. There are two main types of DM:

Type 1 diabetes mellitus (T1DM), which is also referred to as autoimmune diabetes, is caused by the autoimmune destruction of pancreatic β islets **Figure1**, resulting in the loss of these islets or their dysfunction. This ultimately leads to the inability to produce insulin [**14,** [**15**].

Type 2 diabetes mellitus (T2DM) The second type is the most common type of diabetes with over 95% of all diabetes cases globally [**8**]. It is a condition where there is often normal β islet cell but a hyperglycemia is caused by insulin resistance or impairments in insulin secretion [**16**], with the contribution of multiple risk factors such as genetic and environmental factors, obesity and diet [**17,** [**18**].

1.1. Diabetes-related Complications

Complications are common in both type 1 and type 2 diabetes and affect both small and large blood vessels. These complications can be categorized as 'macrovascular disease,' resulting from damage to the arteries, such as cardiovascular disease which affects the heart and blood vessels, and 'microvascular disease' complications caused by damage to small blood vessels. Eye disease (retinopathy), kidney disease (nephropathy), and neural damage (neuropathy) are among the microvascular complications commonly observed in individuals with diabetes [**19,** [**20**].

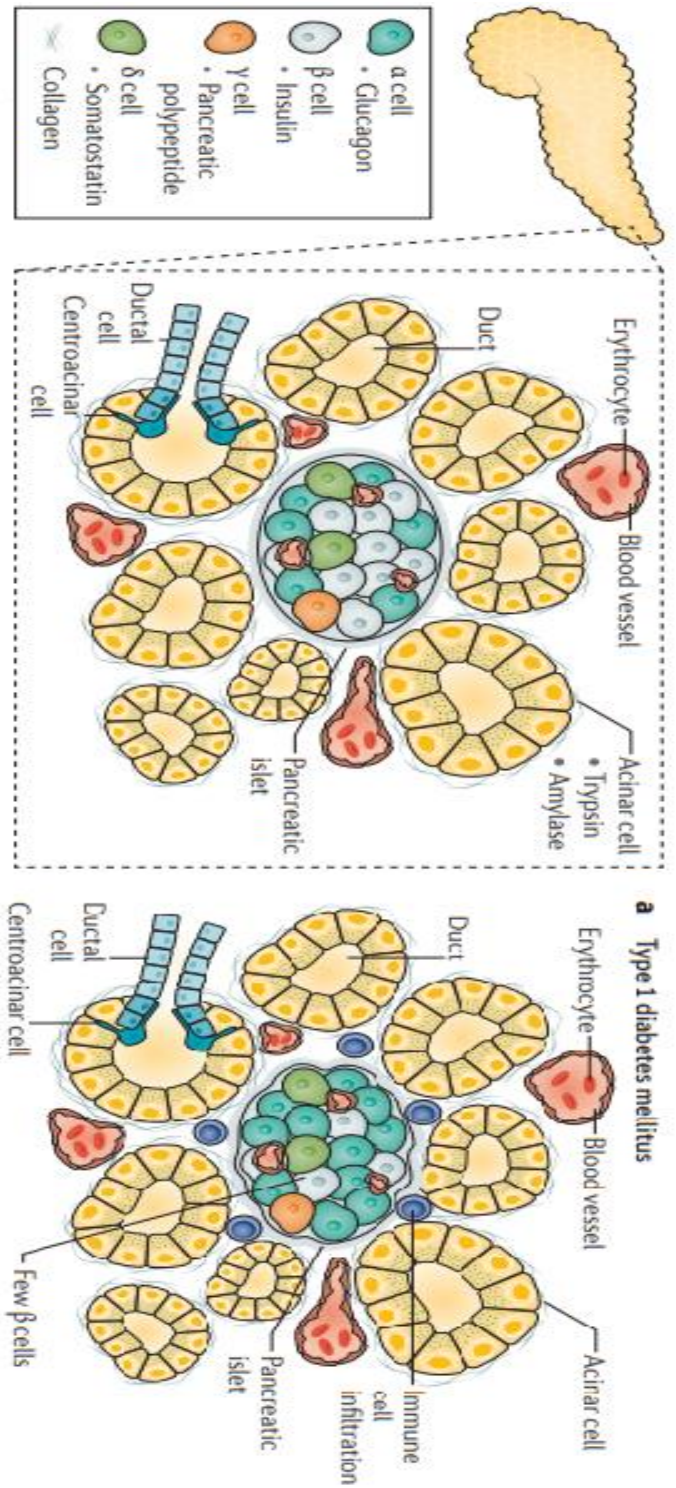


Figure 1. Comparison of pancreatic cell organization in the healthy human pancreas and in subjects with T1DM (a.). The figure highlights the preservation of islet structure in individuals with type 1 diabetes and a reduction in insulin-secreting β cell [**21**].

1.2 Treatment

Various treatment approaches available for managing diabetes summarized in **figure 2**, including lifestyle interventions, pharmacological therapies, and emerging advancements, aiming to effectively control blood glucose levels and improve the overall well-being of individuals with diabetes.

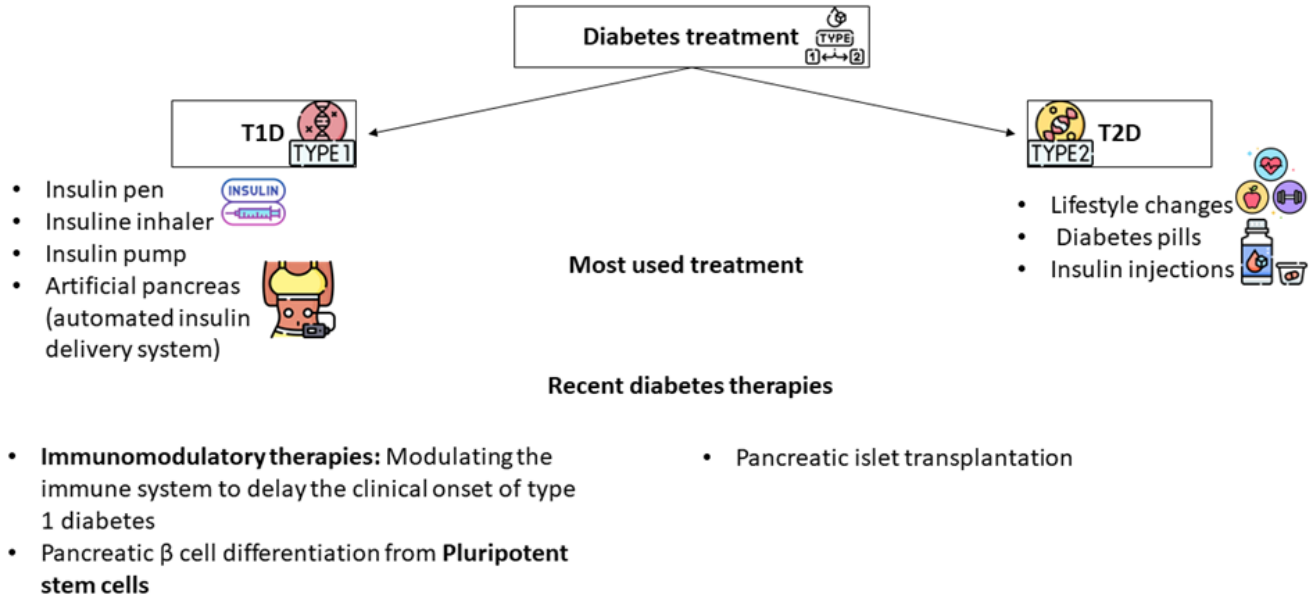


Figure 2. Overview of diabetes treatment: graphical representation [**14,** [**16,** [**22,** [**23**]

1.2.1. Most used

The current treatment options for T1DM focus on the effective administration of exogenous insulin to regulate blood glucose levels and prevent the onset and advancement of diabetes-related complications. The goal is to maintain blood sugar within a safe range that closely aligns with the individual's desired target levels [**23**].

Frequent monitoring of blood glucose levels are also required with the use of the continuous glucose monitoring (CGM) devices or blood glucose meters [**24**].

There are multiple choices available for insulin administration, Multiple daily injection (MDI) insulin therapy typically consists of three or more injections per day and can be administered through insulin syringes, insulin pens [**23**], as well as insulin inhalers and oral insulin [**25**].

Other forms of insulin delivery are also available for diabetes patients to use in the management of their condition. Continuous subcutaneous insulin infusions (CSII) also known as insulin pumps therapy are gaining preference over multiple-daily injections (MDI). Insulin pumps are medical devices that deliver insulin continuously through a catheter placed under the skin, and they require user input to calculate and adjust insulin doses based on blood glucose readings [**26,** [**27**].

Other medical device that uses advanced technology to automatically control blood glucose levels in individuals with T1DM is the artificial pancreas, mimicking the function of a healthy pancreas as **Figure 3** shows. The device typically consists of three components: a continuous glucose monitor (CGM) to measure blood glucose levels, an insulin pump to deliver insulin, and a control algorithm that uses the CGM data to determine insulin doses in real-time [**23**].

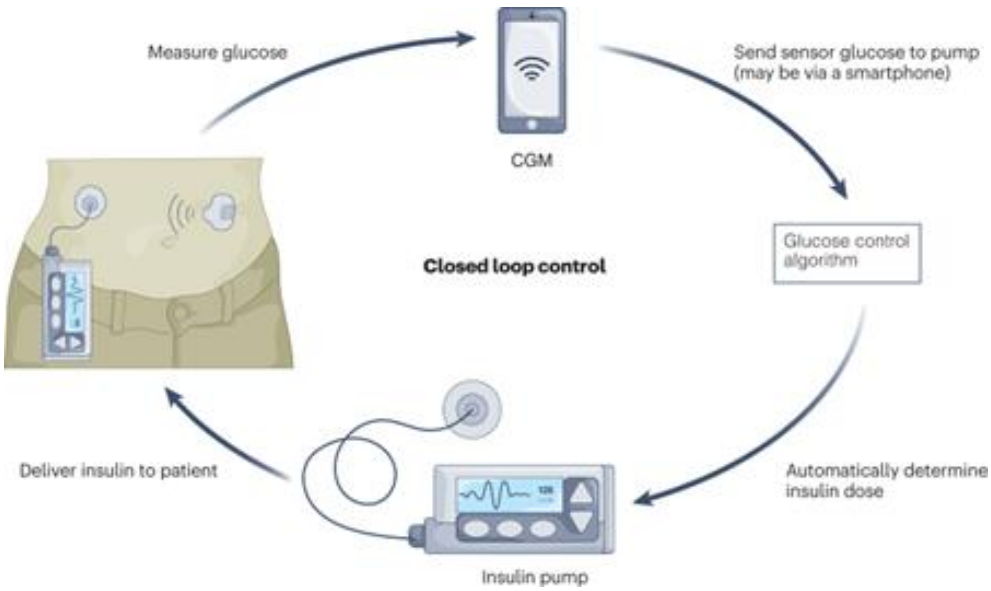


Figure 3. Automated insulin delivery system (artificial pancreas) [**28**].

The treatments for T2DM aim to maintain glycemic control, in order to prevent instability characterized by rise and fall in blood glucose levels, which can lead to complications [**16**].

Effective management of T2DM involves multiple strategies, including making lifestyle changes, using medication as prescribed, and regularly monitoring blood glucose levels [**16**].

The use of insulin in the treatment of T2DM is a common therapeutic approach when oral medications (shown in **Table 1)** and lifestyle interventions fail to maintain adequate blood glucose control [**28**].

Table 1. Commercially available antidiabetic drug classes and their mechanism of action.

Drug class	Mechanism of action	Reference
Biguanide	Enhancing the body's reaction to insulin, reduce hepatic glucose production (decrease gluconeogenesis and stimulate glycolysis)	[**13**] [**29**]
Sulfonylureas	Insulin secretagogues Increase the secretion of insulin from the pancreatic beta-cells	[**30**]
Sodium glucose co-transporter 2(SGLT2) inhibitors	inhibits the reabsorption of glucose and promotes its elimination through the urine.	[**31**]
Thiazolidinediones: Insulin Sensitizers	PPARγ activation reducing insulin resistance Improves insulin sensitivity	[**30,** [**32**]
Incretin mimetics DPP-4 inhibitors; GLP-1 receptor agonists	DPP-IV inhibitors prevent the degradation of GLP-1 Decrease glucagon release and increase insulin secretion	[**13,** [**33**]
Alpha-amylase and alpha-glucosidase inhibitors	reduction in postprandial hyperglycemia inhibition of the digestion of starch into glucose reduction in the release of glucose.	[**10**]

1.2.2. Promising therapies for diabetes

Novel treatment options are currently being developed and could potentially bring change and improve our ability to manage blood sugar levels in individuals with diabetes.

- **Modulation of the immune system**

Immunomodulatory therapies are an important immunotherapeutic approach for the treatment of diabetes **figure 4**, particularly T1DM which results from T-cell-mediated and autoimmune destruction of insulin-producing β cells.

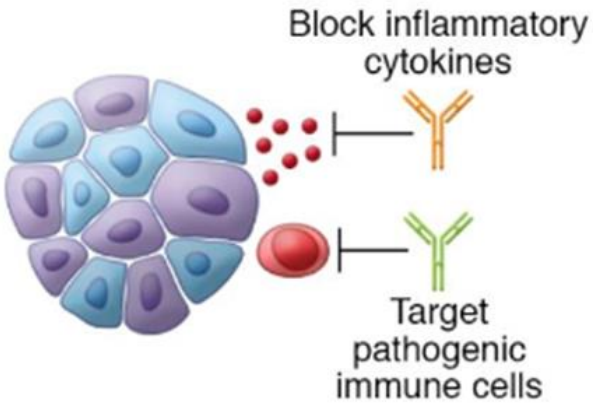


Figure 4. Immunomodulatory approaches to prevent damage or loss of β cells [**34**]

One example of such a therapy is the newly approved drug teplizumab, which can delay the progression of the disease [**22,** [**35**].

- **Pancreatic islet transplantation**

Islet transplantation provides a viable solution for treating T1DM patients through β cell replacement therapy and can result in insulin independence [**36**].

There are three types of islet transplantation procedures: transplantation from cadaveric human pancreas [**37**], allogeneic islet transplantation [**38**], and auto transplantation [**39**].

- **Pancreatic β cell differentiation from pluripotent stem cells**

The use of Human pluripotent stem cells (hPSCs) also offers a hopeful approach for producing functional β cells, which can replace damaged or lost cells in the pancreas. These stem cells are characterized by their unique ability to self-renew, meaning they can divide indefinitely (also known as infinite proliferative capacity), and to transform into any type of cell in the human body [**40**]. Since the discovery of induced pluripotent stem cell (iPSC) technology in 2006 [**41**], several protocols have been developed for differentiating human pluripotent stem cells (PSCs) outlined in **figure 5**, including both embryonic and iPSCs, into pancreatic beta islets in vitro, with the aim of creating a renewable source of functional beta cells for treating diabetes **[15]**.

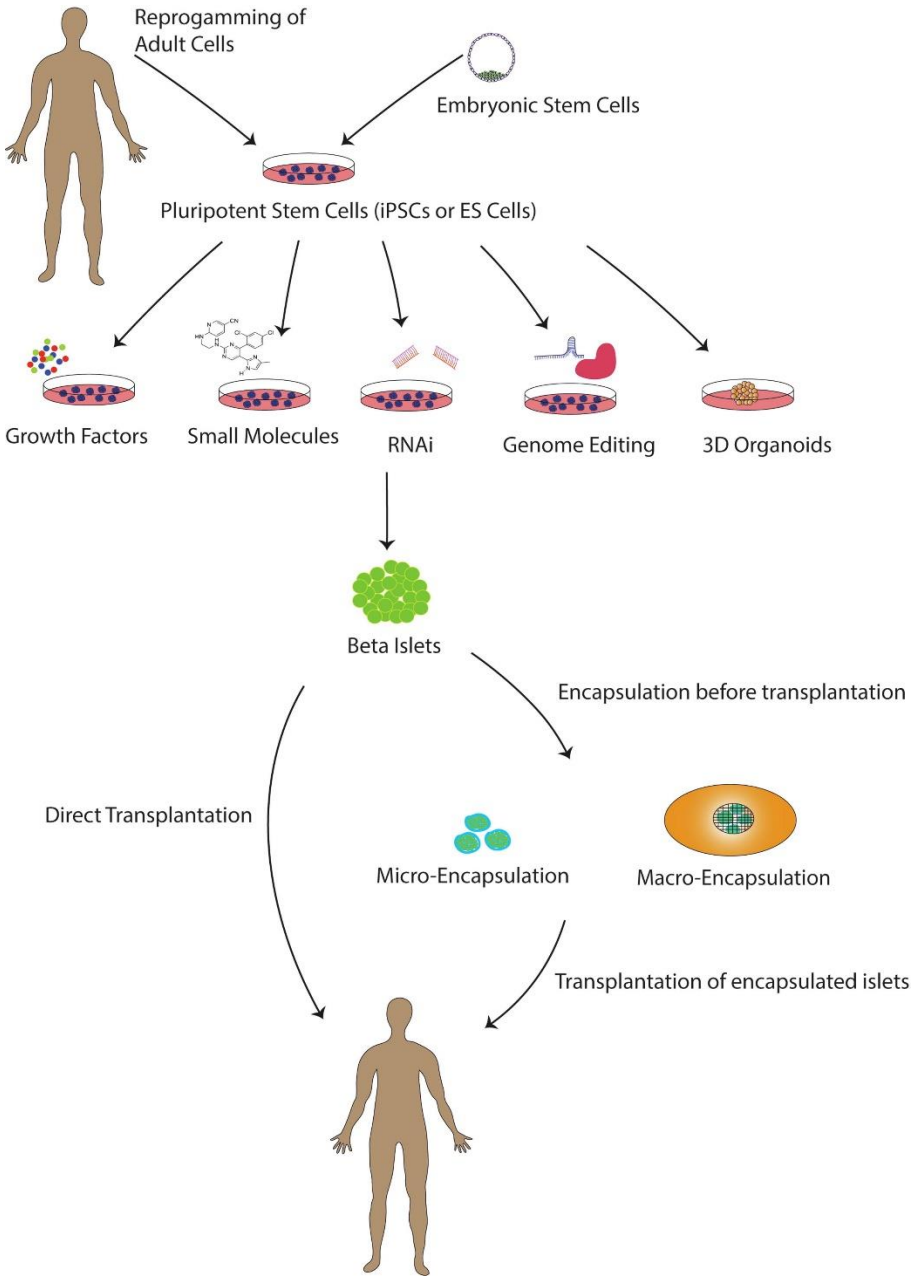


Figure 5. Illustration provides a visual overview of the different protocols and approaches used in generating functional pancreatic β islets from pluripotent stem cells **[14]**

2. Alpha amylase

Alpha-amylase is an enzyme produced in both the salivary glands and pancreas that plays a crucial role in the digestion of carbohydrates in the human body. This enzyme hydrolyzes starch, which is a primary and essential source of dietary carbohydrates for humans, from the mouth all the way to the small intestine, breaking it down into disaccharides and oligosaccharides such as maltose [**9,** [**10**].

Human pancreatic alpha amylase is a 56 kDa protein constituted by 496 amino acids residues in a single polypeptide chain that is structured into three distinct domains [**42**] **figure 8**.

The construction of domain A involves two segments of the polypeptide chain, namely residues 1-99 and 169-404, which are combined to form the domain. **Asp197**, **Glu233**, and **Asp300** are three crucial active site residues involved in the catalytic mechanism [**43**]. **TRP59** plays a crucial role in the substrate binding site of α-amylase and contributes through hydrophobic interactions [**44**]. These residues are situated within domain A [**43**]. Domain B is responsible for binding a calcium ion and appears to be essential for maintaining the structural stability of an active site loop. It is composed of amino acid residues 100 to 168. Domain C is composed of residues 405-496 and it is presumed to play a functional role in the process of binding starch [**42**].

Inhibiting alpha-amylase can slow down carbohydrate digestion, delay glucose uptake, and ultimately reduce blood sugar levels. Currently, there are various commercially available drugs in the market that act as enzymatic inhibitors for alpha-amylase, such as acarbose, miglitol, and voglibose [**10**]. These drugs have been shown to effectively target alpha-amylase.

Despite their benefits, these medications are also reported to have some side effects such as abdominal discomfort, diarrhea, and flatulence [**9,** [**11**].

3. Studied plant:

***Cupressus sempervirens* L.**

Traditional medicine has relied on plants for their medicinal properties, leading to the discovery of important drugs still in use today [**45**].

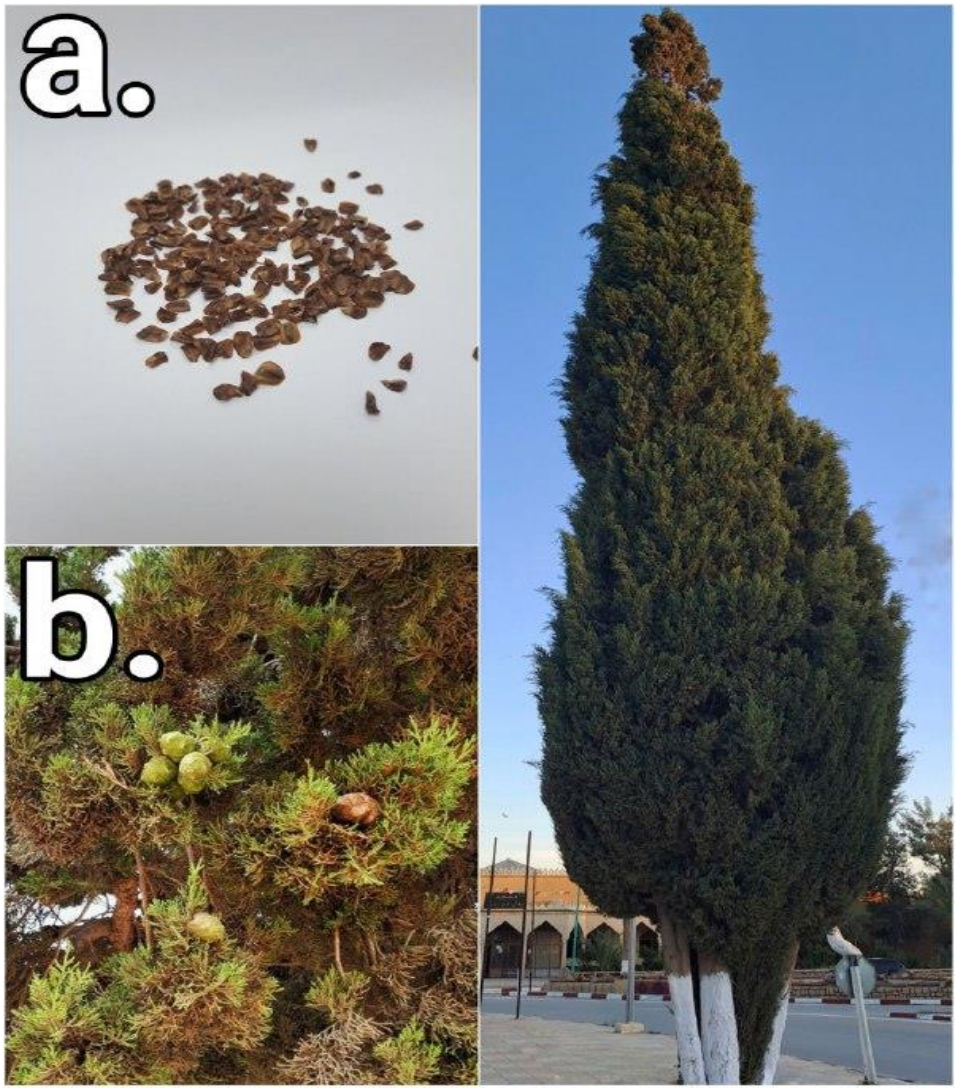


Figure 6. *Cupressus semprevirens* L.

a. its seeds; b. its leaves and cones (original 06-2023)

3.1. Botanical description

Cupressus sempervirens L. also known as Mediterranean cypress/Italian cypress [**46**], an evergreen tree that grows up to 20-30m height with dark green scale-like leaves and male and female elliptical cones **figure 6**. Its shiny brown seeds are contained in ovuliferous scales (8–20 to each fertile scale) [**47,** [**48**].

3.2. Taxonomy

The taxonomy of *Cupressus sempervirens* L. classifies it under the Kingdom Plantae, Division Tracheophyta, Class Pinopsida, Order Pinales, Family Cupressaceae, Genus Cupressus, and Species sempervirens, highlighting its taxonomic position within the plant kingdom as summarized the **figure 7** [**46**].

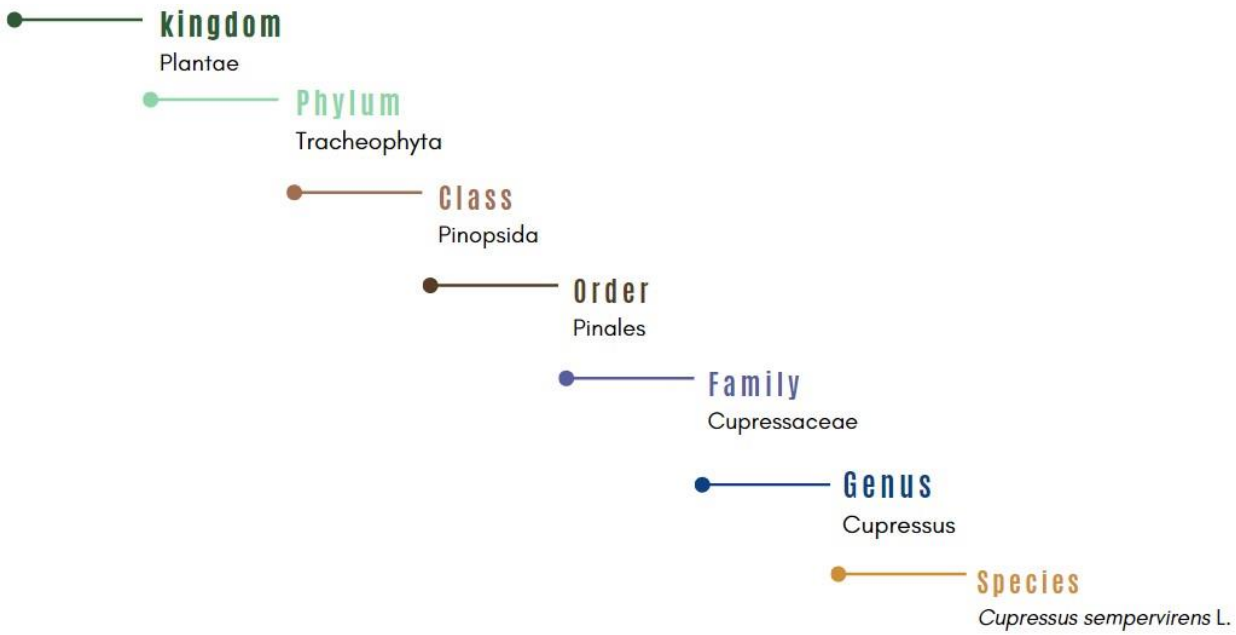


Figure 7. Classification of the plant *Cupressus sempervirens* L. [**46**]

3.3 Geographical distribution

Cupressaceae, is the only family of conifers with cosmopolitan distribution and the third largest gymnosperm family. *Cupressus sempervirens L*, is widely cultivated in the Mediterranean basin, including parts of northern Africa (Algeria, Morocco, Tunisia, Libya, Egypt), western Asia (Palestine, Syria, Jordan, Lebanon, Turkey, Iran, Cyprus), southern Europe (Italy, France, Spain, Portugal), northern and central America as shown in the map in **figure 8 [12]**.

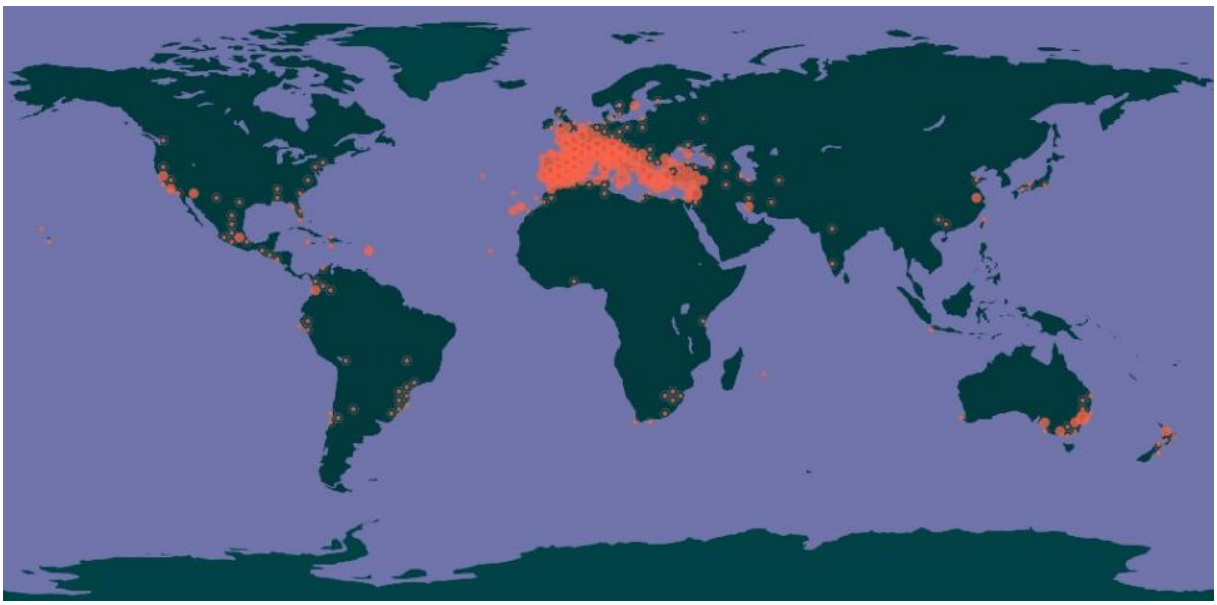


Figure 8. Distribution of the genus Cupressus in the world [**46**]

3.4 Chemical constituents

CS produces diverse secondary metabolites, based on extensive literature survey, the plant presents: alkaloids 0.7%, flavonoids 0.22%, tannin 0.31%, saponins 1.9%, phenols 0.067% and essential oils mainly consist of volatile monoterpenes and sesquiterpenes [**49**].

3.5 Utilization

Cupressus sempervirens has been used for various purposes throughout history. Most Mediterranean nations have incorporated it into their landscape, economy, history, medicine, tradition, and religion.

- Italian Cypress is often planted as an ornamental tree, it is also used for boundary hedges, windbreaks, timber (Cypress wood is highly valued for its durability, strength, and resistance to decay) [**12**].
- In perfumery, cosmetics and soaps, cypress is known for its woody aroma essential oil extracted from the leaves, it is as well used in food industry [**12,** [**50**].
- CS has been used in folk medicine since antiquity, as a diuretic to promote urination and venous circulation, [**49,** [**50**] for headaches, colds, coughs, and bronchitis [**12,** [**50**] for hemorrhoids [**49**], As well in the treatment of diabetes, rheumatism, stomachache, inflammation, toothache, it also possess antiseptic and antimicrobial properties [**12,** [**51**].

EXPERIMENTAL

1. Statistics

Population

The data were obtained from the Algerian Social Insurance Fund, CNAS, and are dated between 2017 and 2023. Data were analyzed using Microsoft Excel 2019.

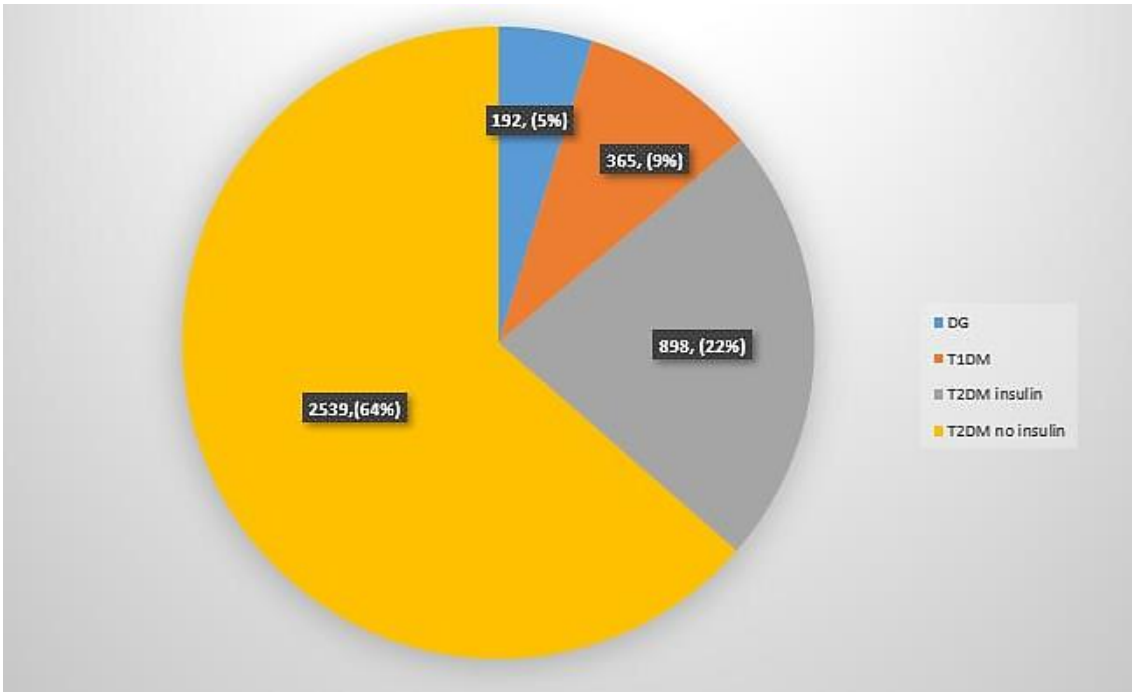


Figure 9. A circle chart demonstrates the total and the proportions of diabetes types.

A total of 3,994 diabetes patients (2,723 females and 1,271 males) were selected **Figure 9**. It is important to note that these patients were diagnosed and confirmed cases by a healthcare provider. Their chronological ages ranged from 1 to 93 years. Of these patients, 365 (9%) were diagnosed with type 1 diabetes, 898 (22%) had type 2 diabetes and used insulin, 2539 (64%) had type 2 diabetes and did not use insulin, and 192 (5%) patients had gestational diabetes.

2. Softwares, databases and web servers

2.1. Softwares and programs

- AutodockTools 1.5.7 (ADT)

ADT is a graphical user interface developed by The Scripps Research Institute, as a part of the AutoDock software package, to prepare protein and ligand files for docking simulations, set up the docking parameters, visualize and analyze the results of docking simulations [**52**].

- AutoDock Vina (ADV)

  ADV is a molecular docking and virtual screening program. This program has proven its efficiency and precision in docking of one or more ligands [**53**].

- Discovery studio visualizer v21.1.0 (DSV)

  DSV is a free, easy-to-use molecular visualization and analysis software developed by BIOVIA, widely used in academic and industrial research, it allows to view and study the shape, size, and chemical properties of molecules by manipulating its 3D structures [**54**].

2.2. Databases

- Protein data bank (PDB)

  PDB is a free database accessible via internet, managed by the Worldwide Protein Data Bank, it is the single global archive of 3D structures data of biological macromolecules (proteins, nucleic acids and complex assemblies) [**55**].

- Pubchem database

  The most used public chemical database as an information resource for biomedical research communities, developed by NCBI (the National Center for Biotechnology Information). The data is organized into multiple data collections, it includes substance, BioAssay, Compound, protein, gene, pathway, Cell Line, taxonomy, and patent [**56**].

2.3. Web servers

- SwissADME

SwissADME is a free web tool of that evaluate the physicochemical properties, drug-likeness and pharmacokinetics of molecules, it enables the prediction of ADME parameters from molecular structure [**57**].

- ADMETlab

ADMETlab is an online platform released in 2018, it provides an accessible and easy to use surface. This webserver offers users the ability to perform multiple analyses and to predict the most ADMET related properties [**58**].

3. Structure-activity relationship (SAR)

We performed a docking study to investigate the main function of the enzyme with various possible inhibitors in order to identify the inhibition mechanism and the specific involved interactions.

Initially, the crystal structure of the alpha amylase (PDB ID: 3BAJ) was obtained from the Protein Data Bank (PDB) [**59**]. The structure is composed of 496 residues in a single polypeptide chain named Human Pancreatic Alpha-Amylase in Complex with Nitrate and Acarbose.

After verifying the docking results by comparing the Energy/RR values with a control dataset, they were visualized using the Discovery studio visualizer v21.1.0 (DSV). The goal was to investigate the amino acids and molecular interactions involved in the binding of the ligand and receptor. This was achieved by identifying the specific amino acids involved in binding and analyzing the interactions formed between them.

Literature references were consulted to acquire data on the phytochemical constituents of *C. sempervirens* L. We selected eight ligands including phenolic compounds and abietane diterpenoid of *Cypress* origin like:

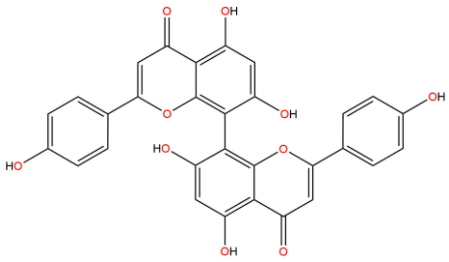
- Cupressuflavone (Mol 1) [**60**];
- Robustaflavone (Mol 2) [**61**];
- Apigenin-4'-glucoside (Mol 3) [**62**];
- Sugiol (Mol 4) [**63**];
- Podocarpusflavone A (Mol 5) [**60**];
- apigenin-7-O-beta-D-glucopyranoside (Mol 6) [**62**];
- Hinokiflavone (Mol 7) [**60**];
- Kaempferol-3-O-rhamnoside (Mol 8) [**64**].

Until now, there are no studies of the antidiabetic effects of the chosen compounds. Therefore, this study evaluated the antidiabetic effect of *C. sempervirens* L. compounds.

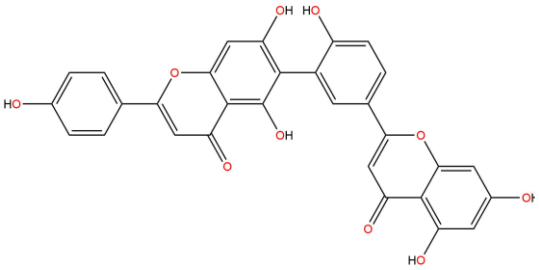
The three-dimensional structure (3D) of the inhibitors were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in SDF format. Then saved in PDB format using Discovery studio visualizer v21.1.0 (DSV). Subsequently, the PDB files were converted to PDBQT form using Auto dock Tools 1.5.7 program to prepare the ligands for molecular docking.

The 2D structures of the eight chemical molecules shown in table 2 were drawn by ChemDraw.

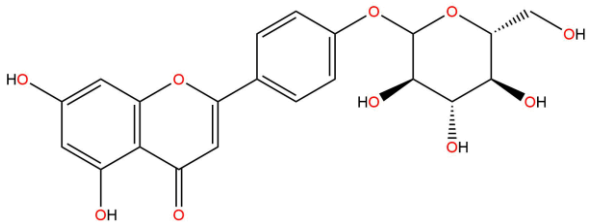
Table 2.The chemical formulas, 2D structures and PubChem CID of the studied molecules.



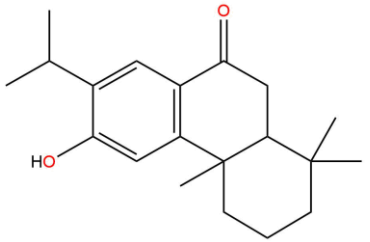
Mol1
$C_{30}H_{18}O_{10}$
PubChem CID 5281609



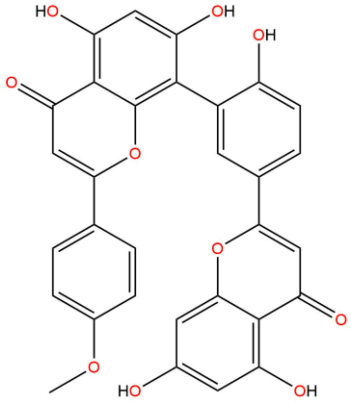
Mol2
$C_{30}H_{18}O_{10}$
PubChem CID 5281694



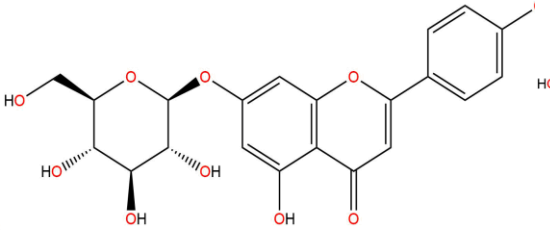
Mol3
$C_{21}H_{20}O_{10}$
PubChem CID 5491384



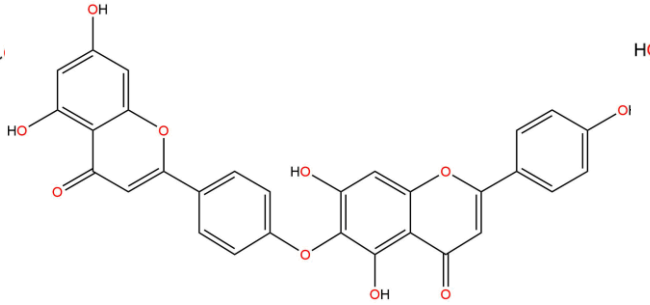
Mol4
$C_{20}H_{28}O_2$
PubChem CID 94162



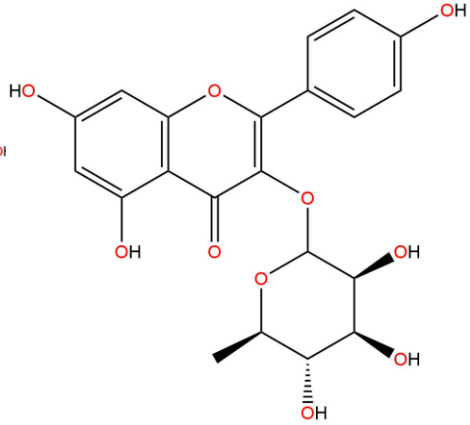
Mol5
$C_{31}H_{20}O_{10}$
PubChem CID 5320644



Mol6
$C_{21}H_{20}O_{10}$
PubChem CID 12304094



Mol7
$C_{30}H_{18}O_{10}$
PubChem CID 5281627



Mol8
$C_{21}H_{20}O_{10}$
PubChem CID 5835713

Mol1: Cupressuflavone; **Mol2:** Robustaflavone; **Mol3:** Apigenin-4'-glucoside; **Mol4:** Sugiol; **Mol5:** Podocarpusflavone A; **Mol6:** apigenin-7-O-beta-D-glucopyranoside; **Mol7:** Hinokiflavone; **Mol8:** Kaempferol-3-O-rhamnoside

3.1 Molecular docking settings

Molecular docking is a well-developed computational technique that uses the structure of molecules to predict their interactions with each other by predicting possible binding modes and estimating binding affinity. This helps in understanding and estimating molecular recognition in terms of both structure and energy. It is widely used in the field of drug discovery [**65,** [**66**].

The preparation of the protein goes through three steps:

- The first step needs to remove everything unnecessary including; water molecules, heteroatoms, ligands and co-ligands, except the required in the active site.
- The second step consists adding polar hydrogens and partial charges to the enzyme structure, this was done using ADT program.
- The third step handling the setting of size and center of the grid box using ADT (x, y and z coordinates). The center of the box is determined with values (x=9.611, y=17.023 and z=41.176) and size (x=22, y=16 and z=18).

After defining the grid box, we have performed the molecular docking using the Auto dock vina.

For docking validation, we redocked the alpha amylase (PDB ID: 3BAJ) with the complexed acarbose **figure10**, the results show fifteen hydrogen bonds between acarbose and alpha amylase as follows: **GLN63**: 2.40 and 2.41 and 3.23Å; **GLY104**:3.07Å; **THR163**:2.02Å; **ARG195**:1.90Å; **LYS200**:1.87 and 2.76Å; **HIS201**:2.49; **GLU233**:2.67and 2.25and 3.31Å; **ASP300**:2.14Å; **HIS305**:3.08Å; **GLY306**:2.51Å. no hydrophobic interactions were found. Acarbose is set as positive control for docking results.

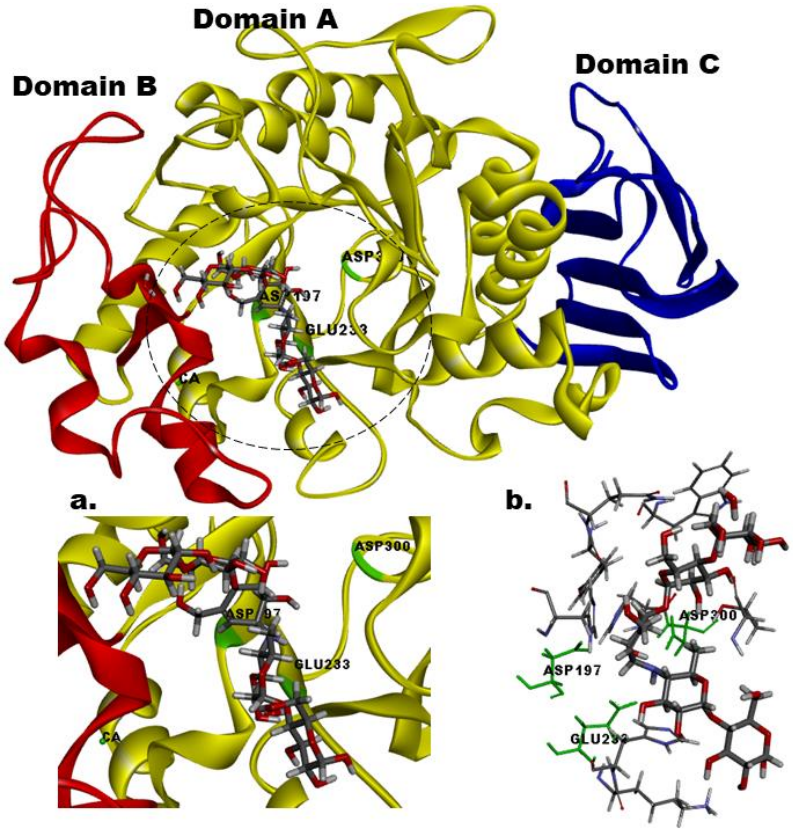


Figure 10. Human pancreatic alpha amylase (PDB ID: 3baj) complexed with acarbose, with Domain A colored in yellow, Domain B colored in red, and Domain C colored in blue.

a. Zooming in on the active site in the 3D structure. b. The ligand is presented in sticks and colored in the default atom color, while the involved amino acids are presented in lines and colored with the default atom color. The catalytic amino acids are colored in green.

4. Absorption, Distribution, Metabolism, Excretion and Toxicity analysis (ADMET)

The first clinical trial is an important step in drug development to ensure the pharmacokinetics properties and avoid failure in the last phases. The predicted, Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) study were analyzed using SwissADME [**57**] and AdmetLab [**58**] as an online servers, webservers enable us to utilize previous *in vitro*/*in vivo* assays from different databases, it offer clinical trials involving small molecules and related bio-assays, as well as features for chemical registration and analysis like NMR, SM, and others [**67**].

In this document, we have outlined some chosen ADMET profile for our compounds:

To check the oral administration bioavailability, the inhibitors were subjected to the drug-likeness Lipinski rule of five [**68**] which is a guideline used in drug discovery.

● The Human intestinal absorption (HIA %): is an important ADME property that refers to the absorption of a compound [**69**]. Predicting intestinal absorption is crucial in accurately determining oral bioavailability [**70**].

● The caco-2 cell permeability: human colon epithelial cancer cell line, is used to predict the absorption of orally administered drugs [**71**].

● The blood-brain barrier (BBB): represent the ability of a compound to reach the brain [**72**].

● The Plasma protein binding (PPB): refers to the biding affinity of a drug to proteins [**73**].

● The toxicity: is an enlargement of the ADME concept, that estimate the potential risks of a compound and understanding its toxicological properties [**74**].

5. Biological activity prediction (PASS)

Prediction of biological activity was done using the PASS webserver. PASS (Prediction of Activity Spectra for Substances) is a webserver designed as a tool that allows us to predict the biological activity and the probable profile for the discovery of new drugs. The predicted activity spectrum of a compound will predict: Pa (probability "to be active") and Pi (probability "to be inactive") [**75,** [**76**].

In order to verify if our chosen compounds exhibit amylase biological activity in the PASS server, we predicted them basing on their SMILES extracted from the PubChem database.

RESULTS AND DISCUSSION

Results

1. Statistical Analysis

The proportion of patients in each diabetes type is:

- Type 1: 365 / 3,994 = 9.14%
- Type 2 with insulin: 898 / 3,994 = 22.48%
- Type 2 without insulin: 2,539 / 3,994 = 63.56%
- Gestational diabetes: 192 / 3,994 = 4.82%

These proportions suggest that type 2 diabetes is more prevalent than type 1 diabetes and that most type 2 patients do not require insulin treatment. Additionally, gestational diabetes affects a relatively small proportion of the diabetes patients in this population.

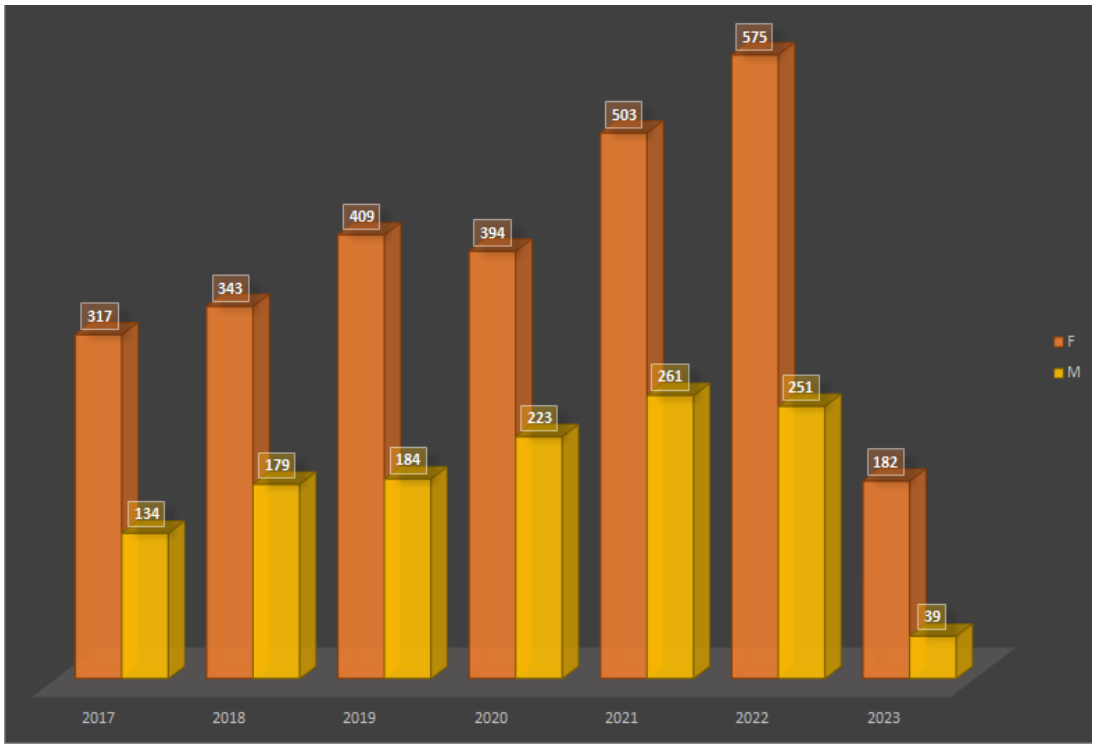


Figure 11. A histogram represents the number of diabetes cases in males and females between 2017 and 2023.

The histogram in **Figure 11** shows a steady increase in the total number of diabetes patients from 2017 to 2023, with a notable spike between 2021 and 2022. This trend is observed across all diabetes types and both genders **Figure 12.**

a. **b.**

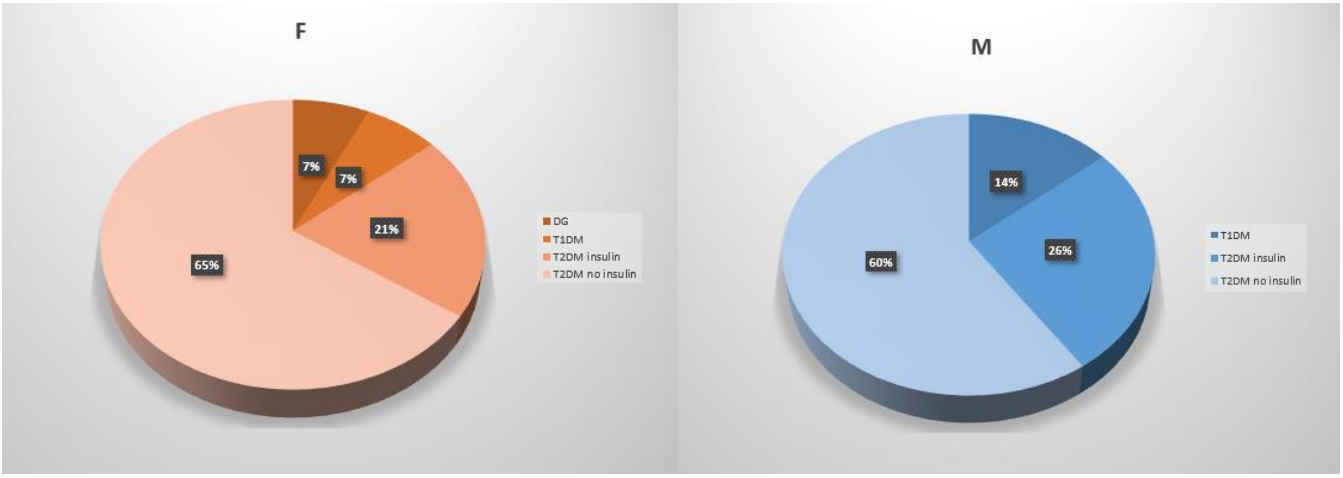


Figure 12. Circle charts represent the proportions of diabetes types by gender. a. Female b. male

One possibility is that the COVID-19 pandemic may have indirectly influenced the increase in diabetes patients between 2021 and 2022 [**77**]. The pandemic was officially declared by the World Health Organization (WHO) on March 11, 2020. However, confirming this hypothesis would require further investigation and data analysis. There are several factors that could contribute to the observed trend, such as changes in healthcare access, lifestyle habits, and environmental factors [**78**].

Overall, the time-series analysis suggests that diabetes is a significant and growing health issue in Algeria, with type 2 diabetes non-insulin dependent being the most prevalent and rapidly increasing type. These findings can inform healthcare policies and interventions aimed at preventing and managing diabetes in Algeria.

2. Structure-activity relationship (SAR) by molecular docking

Depending on minimum energy values and the RR percentage **Mol2/ Mol5** was the best inhibitors among the other tested compounds **table 3**, **Mol3** and **Mol6** were reported as satisfactory inhibitors, and **Mol8** was the last ranked inhibitor model for alpha amylase with the minimum saved criteria comparing to the control.

Otherwise, all the obtained results save **RR percentage of 80% and 100%.** All results are summarized in **Table 3.**

Table 3. The molecular docking results analysis. Catalytic amino acids are colored in red.

Enzyme	Molecule	ΔG Affinity (kcal/mol)	Ki (µM)	RR %	Closest residues	Hydrogen bonds / Length (Å)	Hydrophobic interactions
Wt							
3baj	**Acarbose**	-9.5	0.10	100	GLN63, GLY104, THR163, ARG195, LYS200, HIS201, GLU233, ASP300, HIS305, GLY306,	GLN63 (2.40;2.41;3.23) GLY104(3.07) THR163(2.02) ARG195(1.90) LYS200(1.87;2.76) HIS201 (2.49) GLU233(2.67;2.25; 3.31) ASP300(2.14) HIS305(3.08) GLY306(2.51)	-
	Mol1 (Cupressuflavone)	-9.1	0.21	100	LEU162, LEU165, HIS201, HIS305	HIS201(2.22), HIS305(2.30)	Pi-Alkyl Pi-Sigma
	Mol2 (Robustaflavone)	-11.4	4.30*10^-3	100	TRP59, GLN63, TYR151, LEU162, LEU165, ALA198, HIS201, ASP300	GLN63 (2.37), TYR151(3.00)	Pi-Sigma Pi-Pi Stacked Pi-Alkyl
	Mol3 (Apigenin-4'-glucoside)	-9.2	0.17	100	TRP59 GLU233 ASP300 GLY306	GLU233 (2.25), ASP300 (3.13), GLY306 (3.80)	Pi-Pi Stacked
	Mol4		0.48		TRP59		Pi-Sigma

	(Sugiol)	-8.6		100	TYR62 LEU165	-	Pi-Pi Stacked Alkyl
	Mol5 (Podocarpusflavone A)	-11.6	3.07*10^-3	100	TRP59 LEU162 ASP197 ALA198 HIS201 GLU233 ASP300 HIS305	ASP300 (2.03), ASP197 (2.62), GLU233 (2.40), HIS201 (2.47)	Pi-Sigma Pi-Pi Stacked Pi-Alkyl
	Mol6 (Apigenin-7-O-beta-D-glucopyranoside)	-9.3	0.14	100	TRP59, GLN63, TYR151, LEU165, ARG195, ASP197, ALA198, HIS201, ASP300	GLN63(2.29) TYR151(2.56) ARG195(2.13) HIS201(2.10) ASP300(2.49)	Pi-Pi Stacked Pi-Alkyl
	Mol7 (Hinokiflavone)	-9.35 +/- 0.05	0.13	100	TRP59, GLN63, ILE235, GLU240, HIS305, ALA307	GLU240(1.94) HIS305(3.06) GLN63(2.77)	Pi-Pi Stacked Pi-Alkyl
	Mol8 (Kaempferol-3-O-rhamnoside)	-8.6	0.48	80	TYR62, GLN63, LEU165, ARG195, ASP197, ASP300, HIS305	GLN63(2.30) ASP197(2.10) HIS305(1.84)	Pi-Pi Stacked Pi-Alkyl

3. ADMET analysis

The ADMET properties prediction using SwissADME and AdmetLab servers of tested compounds Mol1-Mol8 showed in **Table 4**

Table 4. ADMET and drug-likeness evaluation of the selected compounds. Green: Low risk Orange: medium risk red: Hight risk

Pharmacokinetics	**Mol 1**	**Mol2**	**Mol 3**	**Mol4**	**Mol 5**	**Mol 6**	**Mol7**	**Mol 8**
			Absorption					
Lipinski Rule of five	Rejected	Rejected	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
Human intestinal absorption (HIA): **+(HIA<30%) -(HIA>30%)**	++	+	++	---	+	++	--	--
Caco-2 permeability (log cm/s) **Optimal: higher than -5.15**	-5.250	-5.259	-5.900	-4.876	-5.19	-5.896	-5.114	-5.962
P-glycoprotein Substrate	Non	Non	Substrate	Non	Non	Substrate	Non	Substrate
P-glycoprotein inhibitor	Non	Non	Non	Inhibitor	Non	Non	Non	Non
			Distribution					
Plasma protein binding (PPB) % **Optimal: <90%**	97.408	98.794	87.983	97.894	96.467	88.240	99.594	91.230
Blood-brain barrier (BBB)	---	---	--	-	---	--	---	---
skin permeability Kp in cm/s	-6.01	-6.01	-8.07	-4.14	-5.86	-7.65	-5.57	-8.07
			Metabolism					
Cytochrome P450 CYP1A2 inhibitor	Inhibitor	Inhibitor	Non inhibitor	Non inhibitor	Inhibitor	Non inhibitor	Inhibitor	Non inhibitor
Cytochrome P450 2C19 inhibition	Non inhibitor	Non inhibitor	Non inhibitor	Inhibitor	Inhibitor	Non inhibitor	Inhibitor	Non inhibitor
Cytochrome P450 2C9 inhibition	Inhibitor	Inhibitor	Non inhibitor	Non inhibitor	Inhibitor	Non inhibitor	inhibitor	Non inhibitor
Cytochrome P450 2D6 inhibition	Non inhibitor	Non inhibitor	Non inhibitor	Inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
Cytochrome P450 2D6 substrate	Non substrate	Non substrate	Non substrate	Substrate	Substrate	Non substrate	substrate	Non substrate
Cytochrome P450 3A4 inhibition	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
Cytochrome P450 3A4 substrate	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate
			Excretion					
$T_{1/2}$<3h	0.543	0.521	0.393	0.091	0.286	0.415	0.735	0.676
			Toxicity					
HERG_inhibition	---	---	---	---	---	---	---	---
less than 50% inhibition: HERG- **more than 50% inhibition: HERG+**	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Ames test	**Non-Mutagen**	**Non-Mutagen**	Mutagen	Non-Mutagen	Non-Mutagen	**Non-Mutagen**	**Non-Mutagen**	Mutagen
Carcinogenicity	Non	Non	carcinogenic	Non	Non	carcinogenic	Non	Non
Hepatotoxicity	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

HERG:Human ether related gene channel

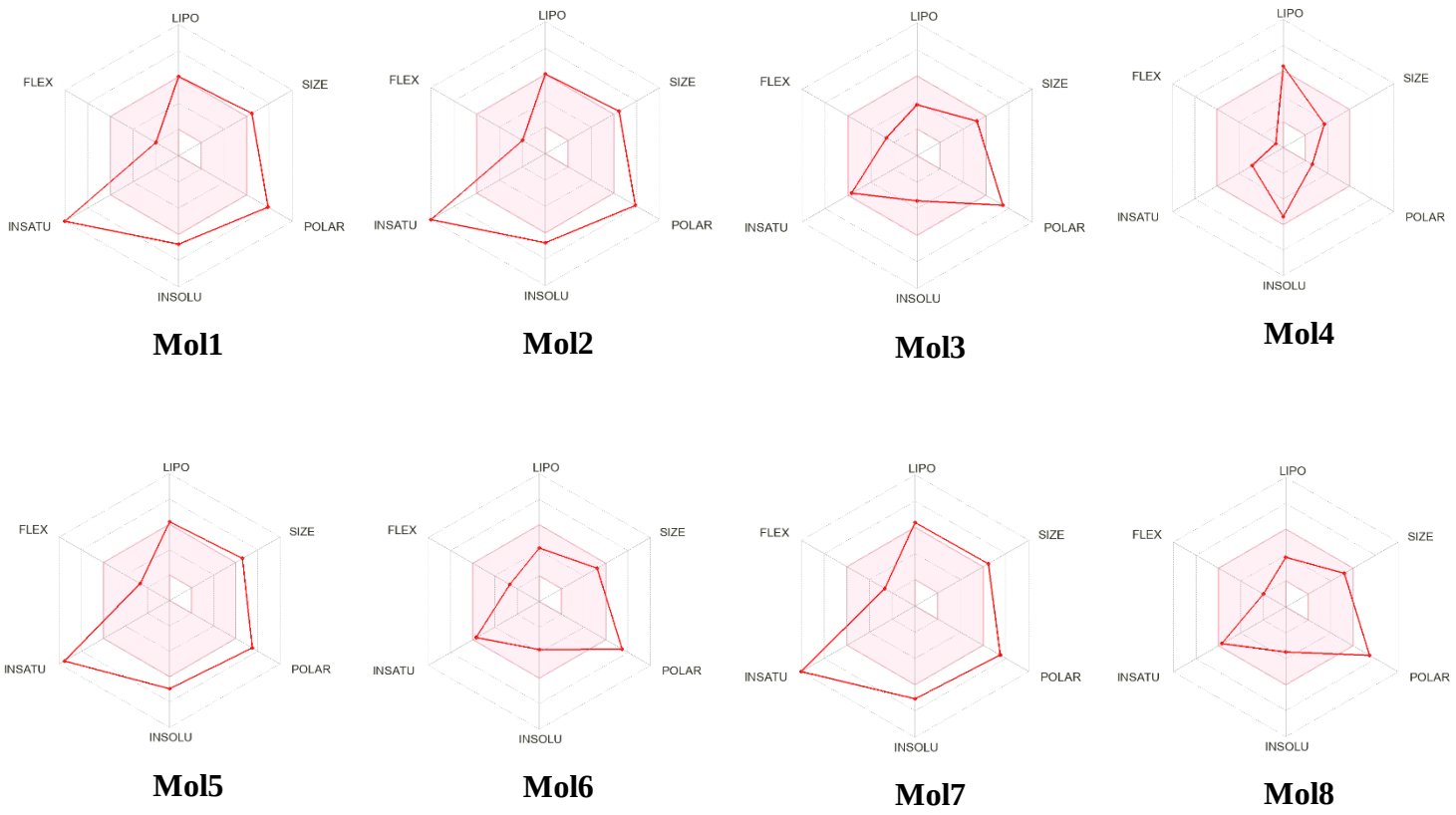


Figure 13. Summary of pharmacokinetic properties of the eight studied molecules. The colored zone is the suitable physiochemical space for oral bioavailability.

Discussion

1. SAR study and molecular docking

Human pancreatic alpha amylase catalyzes the breakdown of polymeric substrates like starch using a double displacement mechanism. In this process, the enzyme initially forms a temporary connection with the starch molecule. Later, with the assistance of water molecules, this connection is broken, leading to the fragmentation of starch into smaller components [**79**]. The nucleophilic amino acid Asp197 plays a crucial role in initiating the formation of the connection. Moreover, the carboxyl groups of Glu233 and Asp300 act as acid-base catalysts, contributing to the breakdown process [**43**]. They facilitate the transfer of protons during the reaction, enhancing the efficiency of the enzymatic hydrolysis.

We studied the phenolic compounds mechanism of action to the Human pancreatic alpha amylase, including the interactions type and the involved amino acids; the predicted results show significant inhibition by the used ligands inside the active site cavity.

Mol1 is the first of the three studied biflavonoid compounds. C–C-type bond between positions C-8 of the two chromene rings. The interaction between Cupressuflavone and human pancreatic α-amylase occurred at amino acid residues in A domain such as His201, and His305, these interactions were sustained through formation of hydrogen bonds with the hydroxyl group of the hydroxyphenyl ring and the chromone ring, respectively. The lack of interaction between Cupressuflavone and the catalytic amino acids suggests that it may not inhibit the digestion of starch carbohydrates.

Mol2 is also a biflavonoid that is formed through the oxidative coupling of two molecules of apigenin. This coupling results in the formation of a bond between position C-3 of the hydroxyphenyl ring and position C-6 of the chromone ring. According to the Md results and from

structural view, Mol 2 was docked in a triangular shape within the enzyme's active site **Figure 14**. We observed that Mol 2 binds to the catalytic site of the enzyme, forming an electrostatic bond with the negatively charged catalytic amino acid ASP300. Furthermore, it establishes hydrogen bonds with two specific amino acids, GLN63 and 151, to interact with the enzyme. Additionally, it forms multiple interactions with hydrophobic amino acids such as LEU162 and LEU165. Notably, TRP59 engages in five hydrophobic interactions of the π-stacked type, which explains its high affinity (-11.4 kcal/mol) to the enzyme. The RR value of Mol 2 was 100%, and its energy ranked second among other inhibitors **Table 3**.

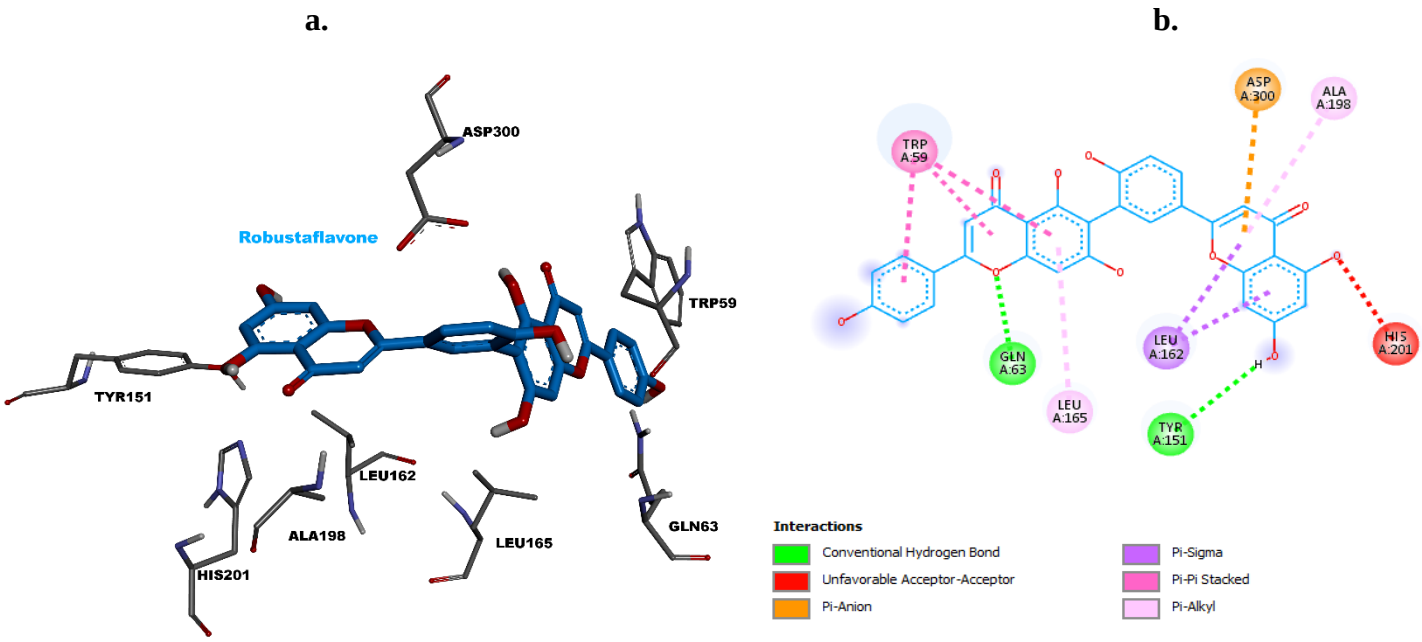


Figure 14. Best docking pose of Mol2 ligand is in blue

a. The molecule 3D structure; **b.** 2D diagram

Mol3 is a glycoside that belongs to the class of organic compounds known as flavonoid O-glycosides; Apigenin 4'-glucoside is a compound in which a glucose molecule is bound to the 4'-position of the apigenin molecule, making it a glycoside of apigenin. the molecular structure of Mol 3 was docked into the active site cavity of the enzyme, based on the results of MD. This explains its affinity to the enzyme, with an RR value of 100%. In the active site of the enzyme, the

hydroxyl groups of the glucose molecule in Apigenin 4'-glucoside form hydrogen bonds with two catalytic amino acids, GLU233 and ASP300. Additionally, a carbon-hydrogen bond is formed with the GLY306 residue. Mol 3 also engages in hydrophobic interaction with the amino acid TRP59, allowing the compound to bind to the enzyme and influence its activity.

Mol4 is an abietane diterpenoid derived of ferruginol. The results show that Mol4 has the least favorable affinity (-8.6 kcal/mol) among the eight studied compounds, with an RR value of 100%. It forms 5 interactions such as TRP59 that forms two hydrophobic interactions π-Sigma type with the benzene ring and the alkyl group, also TYR62 forms two hydrophobic interactions, π-Sigma and π-π stacked. Thus, no conventional hydrogen bond was formed.

Mol5 is an oligomeric flavonoid, a biflavonoid connected through a C –C bond by an aromatic and a none aromatic ring. Md shows that Mol5 best orientation takes a triangular shape **figure 15**, its affinity ranked the first among other inhibitors of **Table 3**, The Gibb's free energy for the alpha amylase- Podocarpusflavone A complex was predicted to be −11.6 kcal/mol, with an RR value of 100%. Mol5 is the only compound that forms conventional hydrogen bonds with the three amino acids of the catalytic site, ASP197, GLU233 and ASP300. Furthermore, hydrophobic interactions were formed between the chromone rings the two amino acids: TRP59 (π-π stacked) and LEU162 (π -Sigma). In total 16 favorable interaction were formed with no unfavorable interaction.

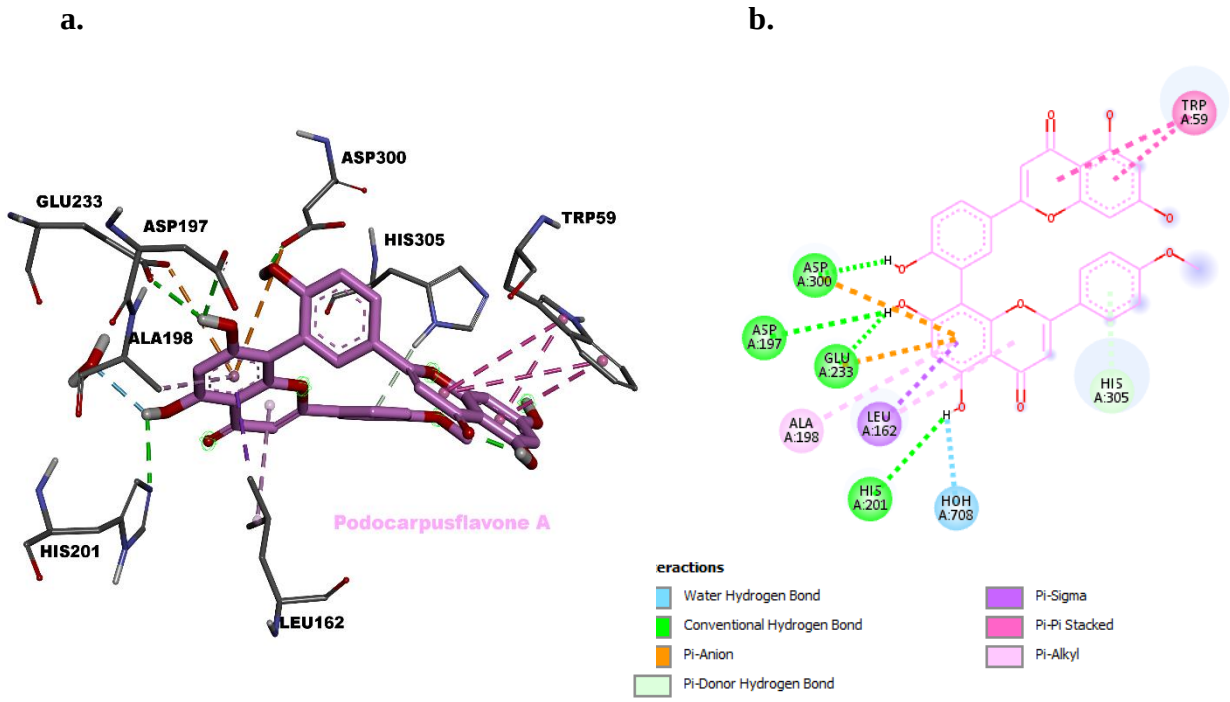


Figure 15 15. Best docking pose of Mol5 ligand is in Purple

a.The molecule 3D structure. **b.** 2D diagram

Mol6 is the second glycoside of apigenin in our study, with a glucose molecule bonded to the 7-position of the apigenin molecule through an O-glycoside bond. According to the docking results, both Mol6 and Mol3 exhibit a similar high affinity to the enzyme, with energies of -9.2 kcal/mol and -9.3 kcal/mol table 3, respectively. Mol6 also binds to the active site in a triangular shape, similar to Mol3. We observed that Mol6 binds to the catalytic site of the enzyme through interactions with two catalytic amino acids. Specifically, it forms an electrostatic bond between its pyran ring and the negatively charged catalytic amino acid ASP197. Additionally, the hydroxyl group of the pyran ring forms a hydrogen bond with ASP300. Mol 6 also engages in hydrophobic interactions with the following amino acids: TRP59, forming two π-stacked interactions, and LEU165 and ALA198, forming two π-alkyl interactions with the benzene rings. Mol6 and Mol3 showed similar ADMET properties.

Mol7 is a biflavonoid with an ether linkage c-o-c between wo connected apigenin units. Also known as bis-apigenyl ether [**80**]. The outcomes of the molecular docking results indicate that Mol7 is one of the three molecules that showed no interaction with the catalytic amino acids. Hinokiflavone was structurally docked in a triangular shape within the subsite of the enzyme's active site. In total, there were 11 favorable interactions. Specifically, two π-π stacked interactions were observed between the indole side chain of the aromatic amino acid TRP59 and the pyran of the chromone ring. The second chromone ring formed four hydrophobic interactions of the π-alkyl type, with two involving ALA307 and two involving ILE235. Additionally, three hydrogen bonds were formed with GLU240, HIS305, and GLN63, respectively. From all the studied compounds, Mol7 exclusively binds to amino acids ILE235, ALA307, and GLU240.

Mol8 is phenolic compound belongs to flavonoids, it is a glycoside of Kaempferol with a 3-O-glycosidic bond. also known as Afzelin [**81**]. From structural view Mol8 was docked in triangular shape and suits the v shape of the active site cavity according to the Md results. Six total Favorable interactions were founded and one unfavorable. Two of the three catalytic amino acids were bonded to the inhibitor. The pyran ring of the Kaempferol formed an electrostatic interaction with the negatively charged ASP300, while a hydrogen bond was established between ASP197 and the hydroxyl group of the pyran ring. Additionally, two other conventional hydrogen bonds were formed: one between GLN63 and the hydroxyl group of the pyran ring, and the other between HIS305 and the hydroxyl group of the deoxy sugar rhamnose. Hydrophobic interactions were also observed, with TYR62 engaging in π-π stacking and LEU162 participating in π-alkyl interactions.

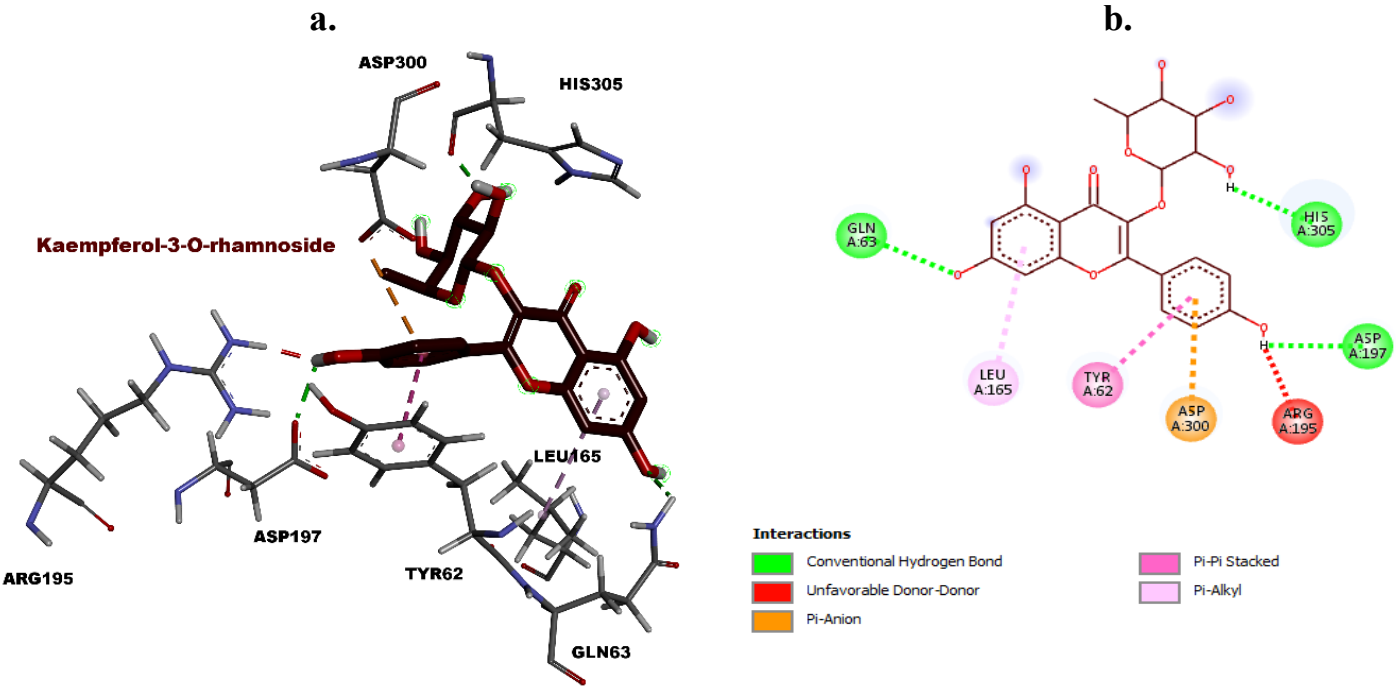


Figure 16. Best docking pose of Mol8 ligand is in brown.

a. The molecule 3D structure**; b.**2D diagram

The RR value of Mol8 was 80%. The presence of Tyr62 exclusively in Mol8 and Mol4 might have affected the substrate orientation through hydrophobic interactions **figure 16.**

2. ADMET Study

Based on the results of the predicted ADMET parameters presented in **table 4** and **figure 13**, the compounds Mol3, Mol4, Mol5, Mol6, Mol7 and Mol8 followed of Lipinski rule while Mol1and Mol2, did not follow in this rule. In the absorption section, Mol4, Mol7 and Mol8 have high human intestinal absorption (HIA) while the rest of the molecules are considered to be poorly absorbed (HIA<30%). Since the primary site of action for alpha amylase inhibitors are within the small intestine, its limited absorption is desirable, by remaining mostly in the intestine, alpha amylase inhibitors can exert their inhibitory effects on carbohydrate digestion locally, without significantly affecting other body tissues or causing systemic side effects. However, the absolute oral bioavailability of a compound depends on both its absorption and metabolism [**70**]. Therefore,

molecules with low absorption rates may require specific semisynthetic modifications or specialized formulations to enhance their absorption and achieve the desired oral bioavailability level [**82**]. All molecules have low blood brain barrier penetration. Regarding distribution and metabolism, it is notable that among the studied compounds, Mol3, Mol6, and Mol8 are identified as P-glycoprotein (P-gp) substrates and are the only ones with a glycosidic bond (O-glycosidic bond). Additionally, it is predicted that these molecules do not inhibit any of the cytochrome P450 enzymes. The eight studied compounds were indicated to be non-hepatotoxic and non-carcinogenic, except Mol3 and Mol6 (carcinogenic).

3. Prediction of biological activity

The prediction of biological activity with the PASS server tool is showed in in **Table 5**.

Table 5. The predicted biological activity for the chosen compounds.

Molecules	Pa	Pi	PBA
Mol 1	0.091	0.042	Isoamylase inhibitor
	0.089	0.052	Beta-amylase inhibitor
	0.136	0.103	Alpha-amylase inhibitor
Mol 2	0.069	0.060	Isoamylase inhibitor
Mol 3	0.706	0.003	Beta-amylase inhibitor
	0.537	0.004	Alpha-amylase inhibitor
	0.501	0.003	Isoamylase inhibitor
Mol 6	0.706	0.003	Beta-amylase inhibitor
	0.537	0.004	Alpha-amylase inhibitor
	0.501	0.003	Isoamylase inhibitor
Mol 8	0.357	0.021	Alpha-amylase inhibitor
	0.249	0.014	Isoamylase inhibitor
	0.188	0.017	Beta-amylase inhibitor

All these molecules show a higher probable activity (Pa) value than probable inactivity (Pi) and are considered as potential candidates for a specific pharmacological activity.

CONCLUSION

Type 2 diabetes mellitus remains to be one of the prevailing health concerns. Inhibiting the digestion of the consumed carbohydrates by suppressing the activity of α-amylase, is one of the used therapeutic approaches to control postprandial hyperglycemia. The treatments rely on medications that are reported to have side effects.

The aim of this study was to investigate, using bioinformatics tools, some *Cupressus sempervirens* L. compounds that may be used to inhibit pancreatic α-amylase and manage hyperglycemia.

The molecular docking results show that Podocarpusflavone A and Robustaflavone have a potential inhibition with a strong binding affinity to pancreatic α-amylase in comparison with acarbose, and a good ADMET profile.

To validate the obtained results, this work can be further advanced and completed through experimental *in vitro* and/or *in vivo* studies. The diverse range of phenolic compounds present in medical plants could help in the discovery of new treatments with more effectiveness and fewer side effects.

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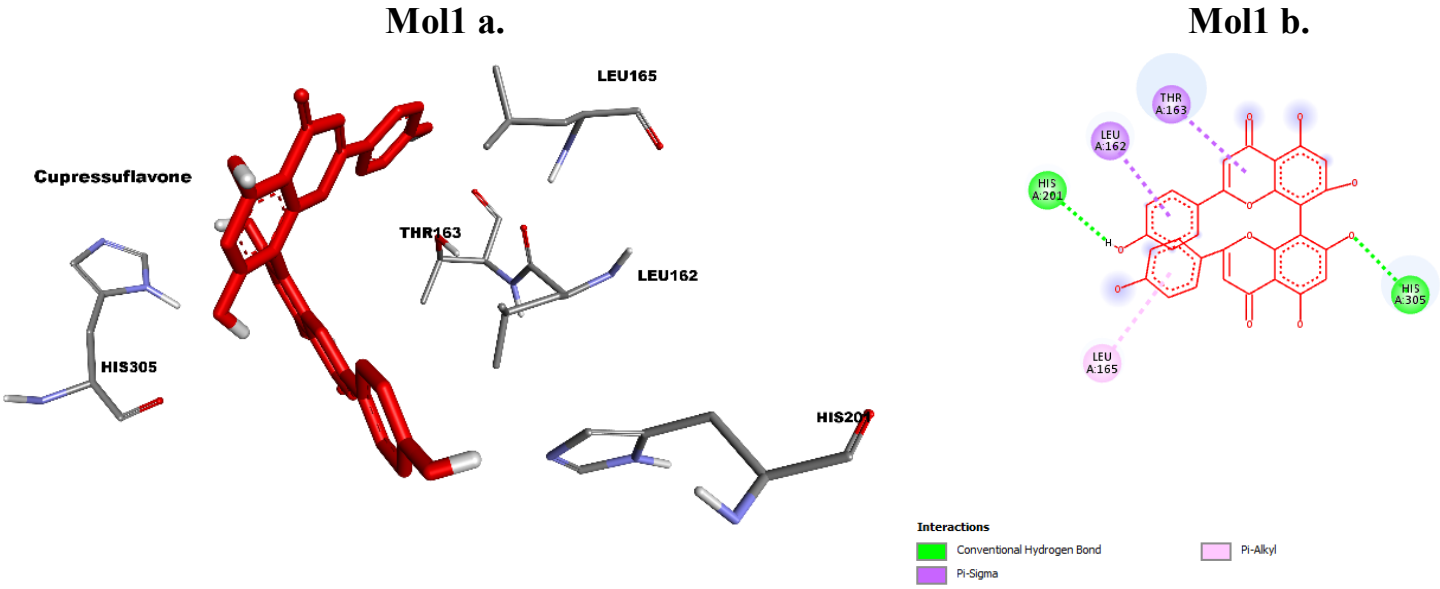
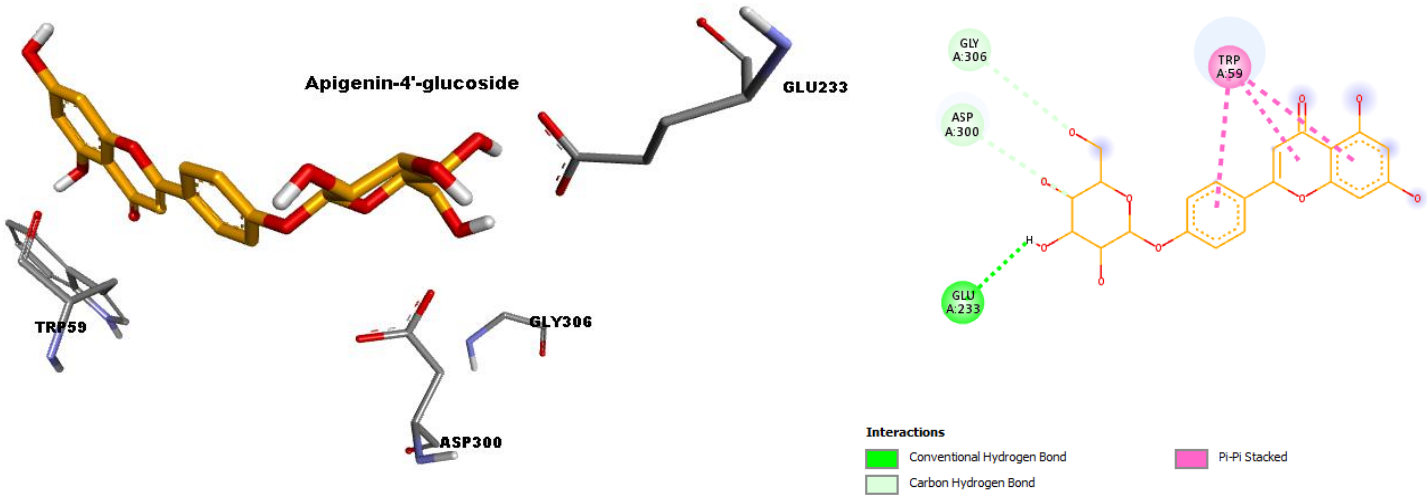
APPENDICES



Appendix 1. Best docking pose of Mol1 ligand is in Red.

The molecule 3D structure. **b.** 2D diagram.

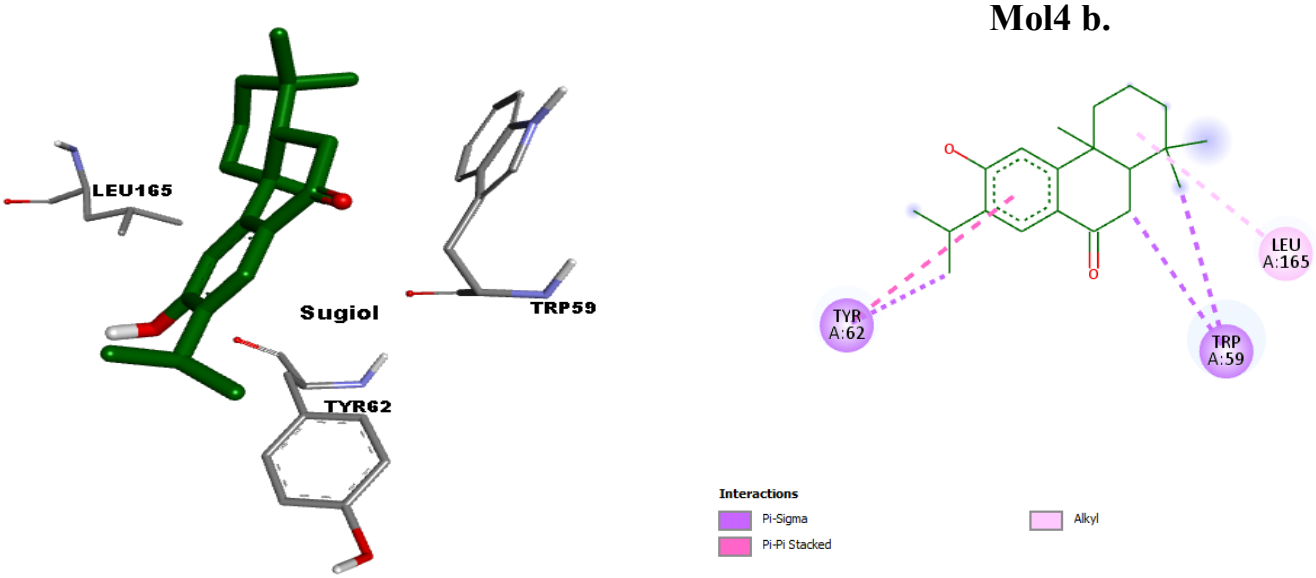
Mol3 a. **Mol3 b.**



Appendix 2. Best docking pose of Mol3 ligand is in Orange

a. The molecule 3D structure. **b.** 2D diagram

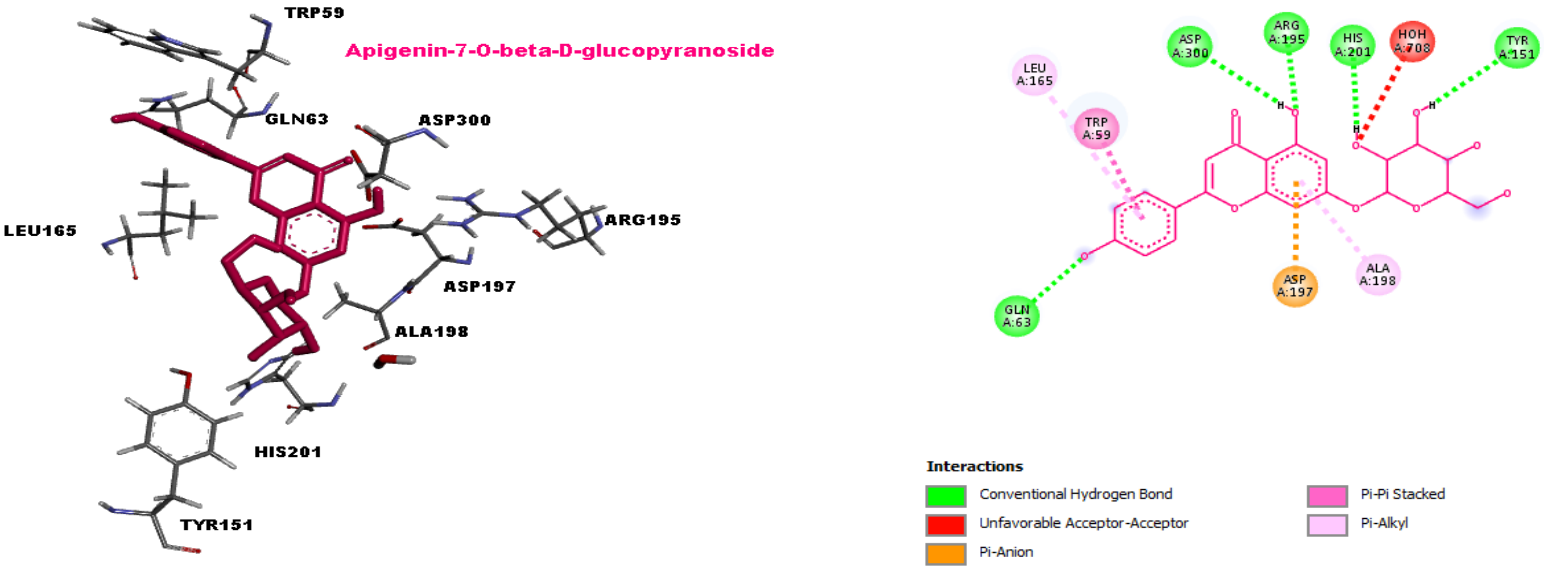
Mol4 a. **Mol4 b.**



Appendix 3. Best docking pose of Mol4 ligand is in Green

a. The molecule 3D structure. **b.** 2D diagram

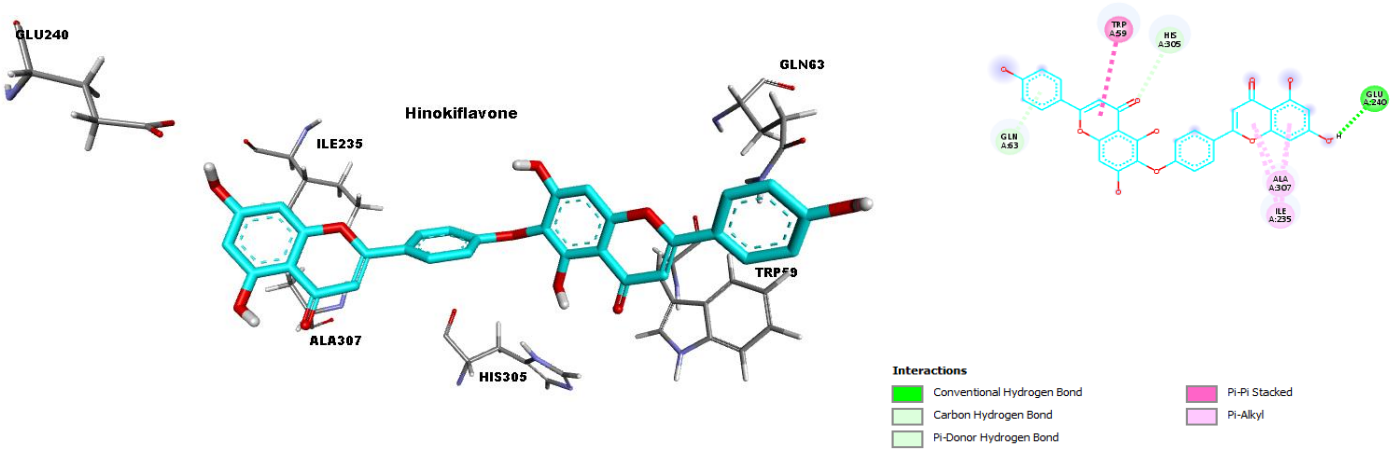
Mol6 a. **Mol6 b.**



Appendix 4. Best docking pose of Mol6 ligand is in Pink

a. The molecule 3D structure. **b.** 2D diagram.

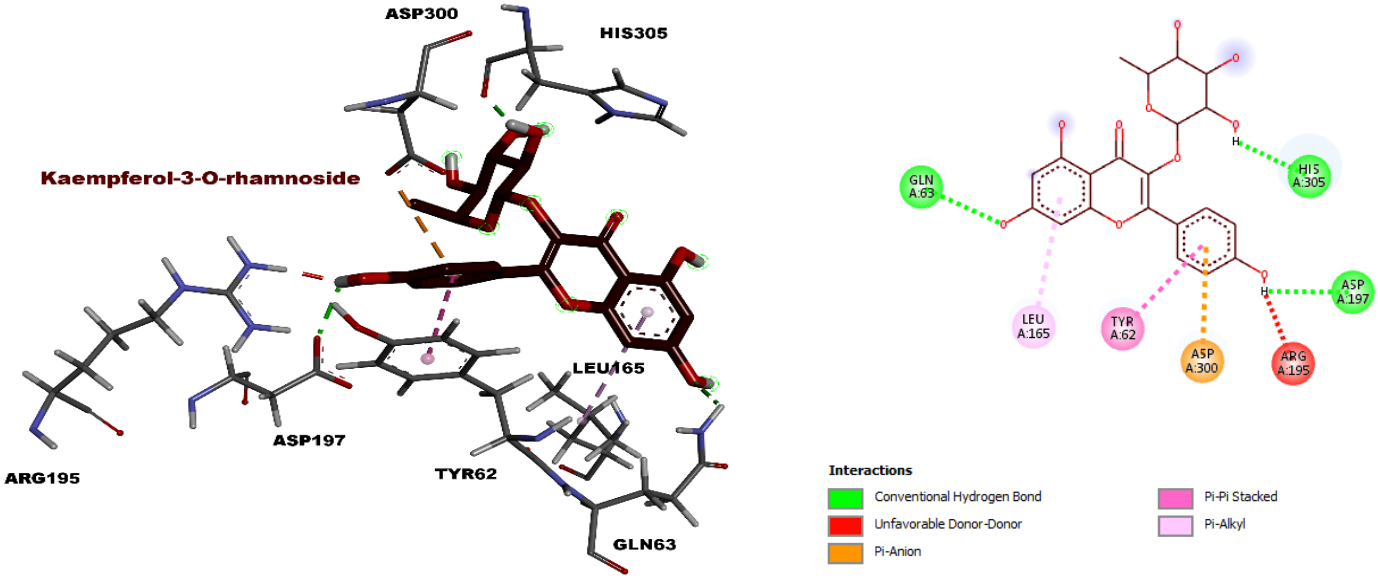
Mol7 a. **Mol7 b.**



Appendix 5. Best docking pose of Mol7 ligand is in light blue

a. The molecule 3D structure. **b.** 2D diagram.

Mol8 a. **Mol8 b.**



Appendix 6. Best docking pose of Mol8 ligand is in brown

a. The molecule 3D structure. **b.** 2D Diagram