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Theme

Exploring the inhibitory effect of some sponges alkaloids on human pancreatic amylase

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Dedication

With a magic feeling of proud, I dedicate this work of my dissertation to

The persons who I am their unique girl daughter, my superhero dad the teacher HARRAT Mahieddine and my pearl of diamond my mom NOUAI Messaouda, who always empower me with their endless love and encouragement, you are my source of inspiration.

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To my beloved grandparents who have passed away, especially my grandfather NOUAI Maamar, who was a mujahid, and HARRAT Mostefa, who was a martyr, rest in peace.

Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder. In this condition of diabetes, the body is unable to store glucose due to a deficiency in the secretion and action of the insulin, resulting in hyperglycemia. The pathophysiology of diabetes is highly complex, making it difficult to find a treatment. The strategy of inhibiting the enzymes involved in polysaccharides degradation provides a promising therapeutic target for type 2 diabetes mellitus (T2DM), in particular the enzyme human pancreatic alpha-amylase (HPA). The medications employed exhibit effectiveness, yet come with associated side effects. The aim of this study is to predict the inhibitory effects of the five marine alkaloids: spongothymidine (ALK1), spongouridine (ALK2), spongiacidin C (ALK3), deoxynebularine (ALK4) and pyridinebetaine A (ALK5), on HPA by an *in silico* study. The study employed a four-step approach, involved: molecular docking: utilizing the AutoDock Vina software, biological activity prediction: using the PASS web server, ADMT analysis: employing the preADMET webserver to assess the absorption, distribution, metabolism, and toxicity (ADMT) properties, and data analysis: using the discovery studio visualizer (DSV) software. The results show that the alkaloids predicted substantial biological activity for HPA, with a favorable ADMT profile. Furthermore, molecular docking revealed that ALK3 identified from *Stylissa massa*, is the most potent inhibitor of HPA, with a binding affinity of -7.5 kcal/mol. The other molecules were found to be moderate inhibitors, while ALK5 was identified as a relatively weak inhibitor. Hence, ALK3 emerges as a potential new candidate in drug discovery for addressing T2DM. As a result, further drug development, encompassing clinical trials, should proceed based on these encouraging findings.

Keywords : Diabetes mellitus, Marine alkaloids, Alpha-amylase, PASS, ADMT, Molecular docking, *Stylissa massa*.

Résumé

Le diabète sucré (DS) est un trouble métabolique chronique. Dans cette forme de diabète, l'organisme est incapable de stocker le glucose en raison d'un déficit dans la sécrétion et l'action de l'insuline, ce qui se traduit par des hyperglycémies. La physiopathologie du diabète est très complexe, ce qui rend difficile la recherche d'un traitement. La stratégie d'inhibition des enzymes impliquées dans la dégradation des polysaccharides constitue une bonne cible thérapeutique pour le diabète de type 2 (DT2), en particulier l'enzyme alpha-amylase pancréatique humaine (APH). Les médicaments utilisés sont efficaces, mais ils sont associés à des effets secondaires. L'objectif de cette étude est de prédire les effets inhibiteurs des cinq alcaloïdes marins: spongothymidine (ALK1), spongouridine (ALK2), spongiacidine C (ALK3), désoxynebularine (ALK4) et pyridinebétaïne A (ALK5), sur l'APH par une étude *in silico*. L'étude a utilisé une approche en quatre étapes, à savoir: l'amarrage moléculaire: avec le logiciel AutoDock Vina, prédiction de l'activité biologique: avec du serveur PASS, analyse ADMT: avec du serveur preADMET pour évaluer les propriétés d'absorption, de distribution, de métabolisme et de toxicité (ADMT), et traitement des données: avec le logiciel discovery studio visualizer (DSV). Les résultats montrent que les alcaloïdes prédisent une activité biologique substantielle pour l'APH, avec un profil ADMT favorable. En outre, l'amarrage moléculaire a révélé que l'ALK de *Stylissa massa* est l'inhibiteur le plus puissant de l'APH, avec une affinité de liaison de -7,5 kcal/mol. Les autres molécules se sont révélées des inhibiteurs modérés, tandis que ALK5 a été identifié comme un inhibiteur relativement faible. L'ALK 3 apparaît donc comme un nouveau candidat potentiel dans la découverte de médicaments pour traiter le DT2. Par conséquent, le développement de nouveaux médicaments, y compris les essais cliniques, devrait se poursuivre sur la base de ces résultats encourageants.

Mots-clés : Diabète sucré, Alcaloïdes marins, Alpha-amylase, PASS, ADMT, Amarrage moléculaire, *Stylissa massa*.

ملخص

إن داء السكري هو اضطراب أيضي مزمن حيث لا يستطيع الجسم تنظيم الغلوكوز بشكل صحيح بسبب نقص في إفراز أو عمل هرمون الأنسولين، مما يؤدي إلى ارتفاع مستوى الغلوكوز في الدم .الفيزيولوجيا المرضية لداء السكري معقدة جدًا، مما يسبب صعوبات في إيجاد علاج نهائي له.لذلك، فإن استراتيجية تثبيط الإنزيمات المتدخلة في هضم السكريات تُعد هدفًا جيدًا لعلاج داء السكري من النوع 2 (دس2)، خاصة إنزيم ألفا أميلاز البنكرياسي البشري(امبب). الأدوية المستعملة فعّالة، لكن لديها آثار جانبية. تهدف دراستنا الى التنبأ في السليكون بالعمل التثبيطي لخمس قلويدات بحرية :سبونجوتايميدين، سبونجويوريدين، سبونجياسيدين س، ديوكسينيبولارين، بيريدينبيتايين ا على امبب. اتبعت الدراسة أربع خطوات شملت: الارساء الجزيئي: باستخدام برنامج اوتودوك فينا، والتنبؤ بالنشاط البيولوجي: باستخدام خادم باص، وتحليل ادمت لتقييم خصائص الامتصاص، التوزيع، الايض، والسمية باستخدام خادم أدميت أولي 2.0، وتحليل البيانات: باستخدام برنامج ديسكوفري ستوديو. اظهرت النتائج أن القلويدات لها نشاط بيولوجي كبير اتجاه امبب، مع خصائص ادمت امنة. بينما كشف الارساء الجزيئي أن سبونجياسيدين س من اسفنجة المانجو هو اقوى مثبط لـ امبب مع قوة ارتباط تبلغ -7.5 كيلو كالوري/مول، كما وجدنا أن المركبات الأخرى هي مثبطات متوسطة، بينما تم تحديد بيريدينبيتايين ا على أنه مثبط ضعيف نسبيًا. وبالتالي يبرز سبونجياسيدين س كمرشح جديد محتمل في اكتشاف الأدوية لمعالجة داء السكري من النوع الثاني. ونتيجة لذلك، ينبغي المضي قدمًا في تطوير الأدوية، بما في ذلك التجارب السريرية، بناءً على هذه النتائج المشجعة.

كلمات مفتاحية: داء السكري، القلويدات البحرية، ألفا–أميليز، باص، ادمت، الارساء الجزيئي، اسفنجة المانجو.

List of figures

List of tables

List of appendices

List of abbreviations

A- B	C-D	E-G
Arg: Arginine **Asp:** Aspartic acid **Asn:** Asparagine **AA:** Amino acid **AGEs:** Advanced Glycation End-Products **AMPK:** AMP-activated protein kinase **AMY:** Amylase **ALK:** Alkaloid **ADT:** AutodockTools **ADV:** Autodock Vina **ADMET:** Absorption, Distribution, Metabolism, Excretion, Toxicity **BBB:** Blood Brain Barrier	**CSII:** Continuous Subcutaneous Insulin Injection **CYPs:** Cytochromes **CD-3:** Cluster of Differentiation **Caco-2:** Human Colon Carcinoma **CGM:** Continuous Glucose Monitoring **cAMP:** cyclic Adenosine monophosphate **DM:** Diabetes Mellitus **DPP-4:** Dipeptidyl peptidase 4 **DNA:** Deoxyribonucleic Acid **DSV:** Discovery Studio Visualizer	**ESCs:** human Embryonic Stem Cells **Gln:** Glutamine **Glu:** Glutamic acid **GIP:** Glucose-dependent insulinotropic polypeptide **GLP-1:** Glucagon-like peptide 1 **GAD:** Glutamic Acid Decarboxylase **GADA:** Glutamic Acid Decarboxylase autoantibodies **GLUT4:** Glucose Transporter 4
H-I	**K-L**	**M-N**
His: Histidine **HLA:** Human Leukocyte antigen **HERG:** Human Ether Related Gene **HIA:** Human Intestinal Absorption **HPA:** Human Pancreatic Amylase **HSA:** Human Salivary Amylase **IDDM:** Insulin Dependent Diabetes Mellitus **IAA:** Insulin Auto-Antibodies **IR:** Insulin Resistance **iPSCs:** induced Pluripotent Stem Cells **IUPAC:** International Union of Pure and Applied Chemistry	**Kcal:** Kilocalorie **KDa:** Kilo Daltons **Lys:** Lysine **Leu:** Leucine **LDL:** Low Density Lipoproteins	**MNA:** Multilevel Neighbors of Atoms **MDCK:** Madin-Darby Canine Kidney **MS:** Mass spectroscopy **NMR:** Nuclear magnetic resonance **NAG:** 2-acetamido-2-deoxy-beta-D-glucopyranose
P	**R**	**S-Z**
PDB: Protein Data Bank **PDBQT:** Protein Data Bank Partial Charge (Q), atom Type **PPAR-γ:** Peroxisome Proliferator-Activated Receptor Gamma **PDF:** Portable Document Format **PASS:** Prediction of Activity Spectra for Substances **P-gp:** Permeability glycoprotein **Pa:** probability for active compound **Pi:** probability for inactive compound **PK:** Pharmacokinetics **PBAn:** Predicted Biological Activity numbers **PAAI:** Pancreatic alpha amylase inhibition	**RNA:** Ribonucleic Acid **RR:** Repetition Ratio **RMSD:** Root Mean Square Deviation	**SDF:** Spatial Data File **Tyr:** Tyrosine **Trp:** Tryptophane **Thr:** Threonine **T1DM:** Type 1 Diabetes Mellitus **T2DM:** Type 2 Diabetes Mellitus **SGLT2:** Sodium-Glucose Transport Protein 2 **ZNT8:** Zinc Transporter 8 **ZNT8A:** Zinc transporter 8 autoantibodies

Table of contents

INTRODUCTION

INTRODUCTION

Diabetes mellitus (DM) is an antiquated disease that has been described in various literatures: Indian, Chinese, and especially in the ancient medical texts of the Egyptians, the Ebers Papyrus; diabetes is described as a polyuria disease **[1]**. The name "diabetes" was described by the Greek scientist Aretaeus of Cappadocia, derived from the Greek word "siphon"**[2]**, then the word "mellitus" was added to diabetes in 1674 by the English anatomist Thomas Willis after his discovery of the sweet taste of urine in diabetic patients, and this was proven by another scientist who boiled patients urine until he obtained a crystalline substance with the same taste as brown sugar **[1, 3, 4]**.

The two scientists Minkowski and Mering discovered that the pancreas was linked to diabetes, and based on these findings, Canadian surgeon Banting and American physician Best discovered and extracted the hormone insulin in 1921, winning the Nobel Prize for their work. At the time, Banting announced: "Insulin is not a cure for diabetes, it's a treatment" **[1, 3, 4]**.

This disease is a very common, and the fastest growing in the 21st century. It is characterized by high blood sugar levels due to a failure of insulin secretion or action, or both, and manifests as thirst, increased appetite, weight loss, and frequent urination. Diabetes can lead to other problems with vital organs, including a number of complications. There are two main types of diabetes: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) **[1, 4, 5]**. Diabetes is predicted to act nearly on 643 million by 2030 **[6]** and 800 million adults by 2045 **[7]**.

Inhibition of carbohydrates digestion is a target methodology used in the treatment of T2DM **[7]**, like pancreatic alpha-amylase inhibition (PAAI), to slow glucose breakdown and reduce hyperglycemia levels in the postprandial period. In the context of PAAI, several drugs are used as antidiabetic agents, such as miglitol, voglibose and acarbose. The latter is a competitive alpha-amylase inhibitor **[7, 8]**.

INTRODUCTION

We selected to marine pharmacognosy, supporting a new strategy using sponge-derived alkaloids to find an effective treatment with fewer side effects. Marine sponges (Porifera) are sessile marine invertebrates that produce unique bioactive secondary metabolites that act as chemical defenses against predators and fouling organisms **[9]**. Marine sponges have shown promising therapeutic potential in various fields, including cancer treatment, as antiviral, antitumor, antifungal and enzyme inhibitors **[9, 10]**.

This study aims to determine new antidiabetic alkaloids by predicting their inhibitory effect on the human pancreatic amylase (HPA) sourced from four sponges: *Tectitethya crypta*, *Stylissa massa*, *Acanthella cavernosa*, and *Agelas dispar*. This work will be achieved through biological activity prediction using PASS, evaluating their pharmacokinetic properties like absorption, distribution, metabolism, and toxicity (ADMT) with pre-ADMET server 2.0, and predicting their binding affinity via molecular docking utilizing AutoDock Vina and AutoDock Tools programs.

The document contained three principal parts organized as follows:

- The initial section offers a concise literature review, summarizing key concepts crucial to this study. Initially, we outline the types of diabetes, their associated complications, and the available treatments. Following this, we delve into the human pancreatic α-amylase, highlighting its significance as a therapeutic target. Furthermore, we explore the taxonomy, distribution, and morphology of the marine sponges utilized in our research.
- The second section focuses on the experimental procedures, detailing the materials and methodologies employed throughout the research. This is followed by a presentation of the obtained results, which are then analyzed and discussed.
- The final part of the document is the conclusion and perspectives.

LITERATURE REVIEW

I. Diabetes mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder, which is characterized by a high blood sugar level **[7, 11]**. It can represent a very serious risk to a patient's life if not diagnosed early and treated properly **[12, 13]**. Moreover, diabetes can be symptomatic and sometimes not manifest at all **[14]**, furthermore people of all ages can be affected by diabetes **[15]**. There are two major types of it:

1. Type 1 diabetes mellitus

The immune system plays a key role in protecting the body against pathogenic substances **[16]**. Type 1 diabetes mellitus (T1DM) **[17]** is an inherited autoimmune disease in which the immune system plays a reverse role, namely self-destructing and self-attacking the beta cells of the islet of Langerhans, which are responsible for insulin production **[16, 18, 19]**, making T1DM **(Figure 2)** an insulin-dependent diabetes mellitus (IDDM) **[20]** which is an essential hormone with a hypoglycemic effect to balance blood glucose levels **[21]**, and the patient has to take exogenous insulin for the maintenance of his body's homeostasis **[22]**. More than 50 genes are responsible for T1DM, And the real process is not well understood **[23, 24]**.

The causes of T1DM can be genetic or environmental: the most studied idea concerning genetic factors and T1DM is the association with human leukocyte antigen (HLA) genes **[17, 23]**, these genes produce HLA proteins responsible for the presentation of exogenous antigens to lymphocytes T (T-cells) **[25]**. In T1DM these proteins undergo genetic variations (mutations) **[24, 26]**, which modify their ability to fix the antigens that will be presented **[24]**. Insulin, GAD, and ZnT8 are some of the autoantigens that could be present on the HLA proteins, which lead to the activation of the immune system (T and B cells) against pancreatic β-cells **(Figure 1)**, by the production of auto-antibodies (AA) against β-cells proteins (AA: IAA (insulin autoantibodies), GADA (glutamic acid decarboxylase autoantibodies), ZnT8A (zinc transporter 8 autoantibodies), also called auto-

antigens **[16, 27-30]**. The actual process by which these forms appear, and their exact causes, are not well understood, and are still under investigation **[16]**. For environmental factors: some viral infections lead to β-cells destruction **[18]**, because some viruses have proteins with similarities to the β-cells proteins mentioned above, and the immune response will be directed against the virus and the self-antigens presented on the HLA proteins **[31]**.

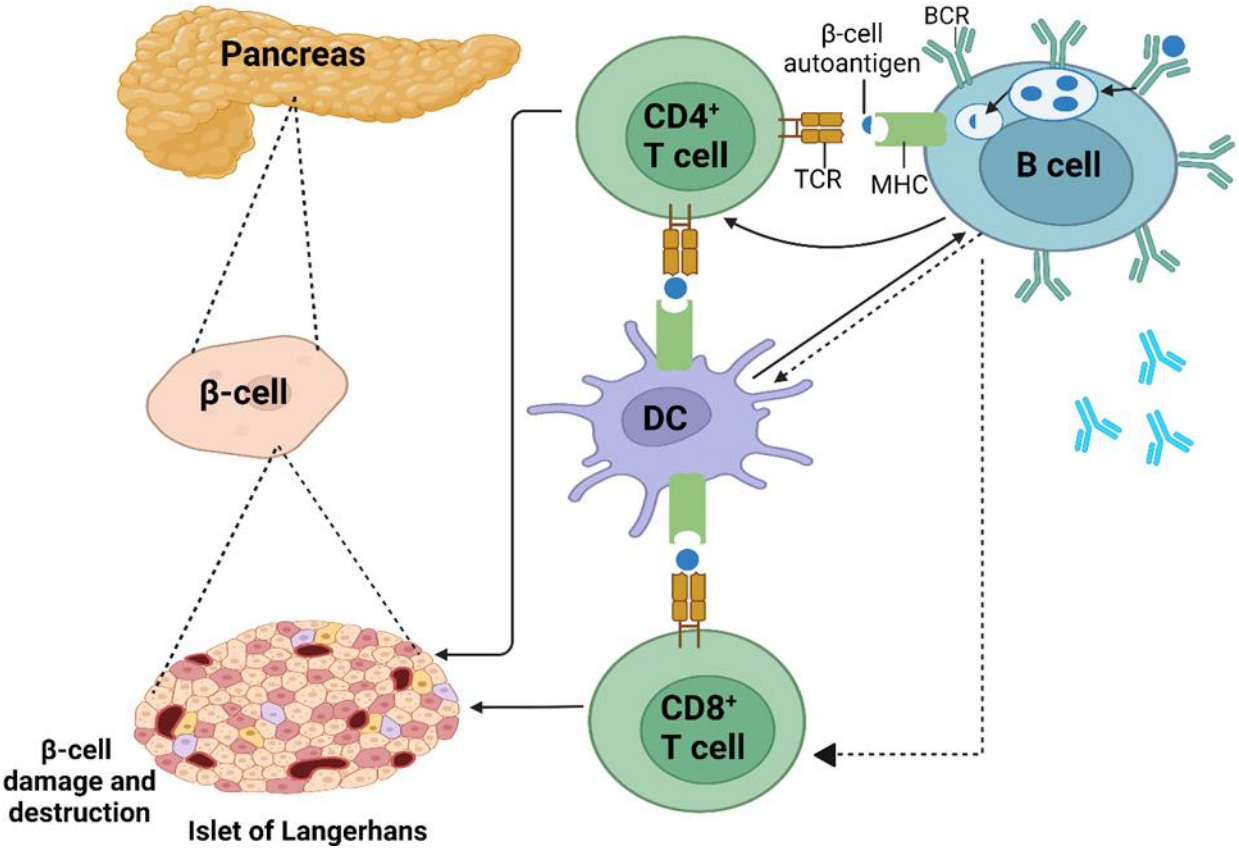


Figure 1. The auto destruction of β-cells by the immune system (T and B cells) **[32]**.

2. Type 2 diabetes mellitus

Type 2 diabetes or T2DM is a type of non-insulin-dependent diabetes, which is very common in the elderly, accounting for 80-90% of cases **[33]**, obese people **[21]**, and it can also affect people after other illnesses that can lead to T2DM like viral infections **[31]**. In the case of type 2 diabetes, either the body does not produce enough insulin to balance blood glucose levels, or it is due to

insulin resistance (IR), or both **[21, 34]**, in which the body doesn't respond to insulin and consequently the level of sugar in blood still elevated **[34, 35]**. The insulin resistance is caused because of a mutation in insulin receptor **[33, 36, 37]** or a mutation in insulin gene **[38]**: In the first case, the receptor has been unable to recognize and bind insulin, and in the second, the structure of insulin has been altered, so the hormone exists but its activity and effect don't **(Figure 2)**.

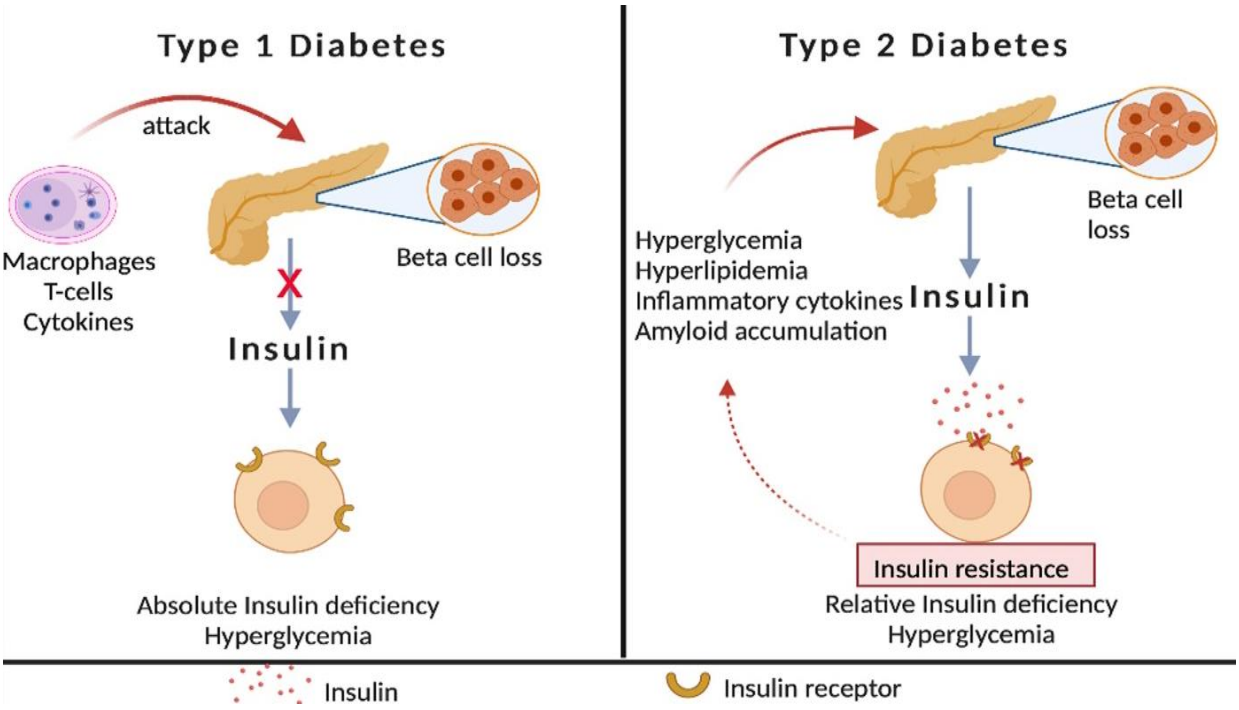


Figure 2. The difference between type 1 and type 2 diabetes mellitus **[39]**.

3. Diabetes-related complications

Diabetes can cause dangerous and life threating problems **[40]**. The high level of glucose in the blood is the main risk of diabetes complications, these complications can be microvascular such as: kidney failure (nephropathy), neurons damage (neuropathy) or eyes problems (retinopathy), or macrovascular like: cardiovascular disease which is a serious problem caused by diabetes **[41, 42]**. The high level of sugar can lead to:

- The non-enzymatic glycosylation of proteins and lipids which leads to the formation of AGEs (advanced Glycation End-Products), which damage, unbalance blood vessel cells.
- Free radical synthesis due to glucose autoxidation.
- Activation of the polyol pathway, leading to the production of alcoholic compounds that accumulate in cells.
- Macrovascular complications: the main example is the atherosclerosis through the non-enzymatic glycosylation of low-density lipoprotein (LDL) cholesterol other apolipoproteins, and blood clotting factors.
- Glucose can directly damage the endothelium or atherosclerotic plaque of blood arteries, and leading to a severe and killer cardiovascular disease **[41-44]**.

4. Diabetes treatment

Numerous strategies are used to treat T1DM and T2DM. To date, there is no cure for diabetes, only treatment to control and balance blood sugar levels and avoid hyperglycemia **[45-47]**.

4.1. Type 1 diabetes mellitus

- **Injected insulin**

The insulin could be injected by using: specifics syringes and vials, a 3 or 4 times per day in this case the patient is able to use them alone and sometimes he needs to mixt several insulin preparations to obtain the biological effect, or also using insulin pens, by this tool the insulin holder and the syringe are coupled in one device, the insulin pens are easy to use than syringes and give a better glycemic control, also they allow to adjust the insulin dose automatically **[48, 49]**.

Another device used is the insulin pump, or also called a continuous subcutaneous insulin injection (CSII), in the pump exist an insulin container connected to a subcutaneous catheter by a plastic tubing and sometimes without it, in this case we call the pump: patch pump which is controlled

using wireless device, the catheter needs to be changed each two or three days, theses pumps give a more comfortable lifestyle for patients **[48, 50]**, in all cases the patient has to control the level of glucose in his blood by using the blood glucose meter **(Figure 3) [51]**.

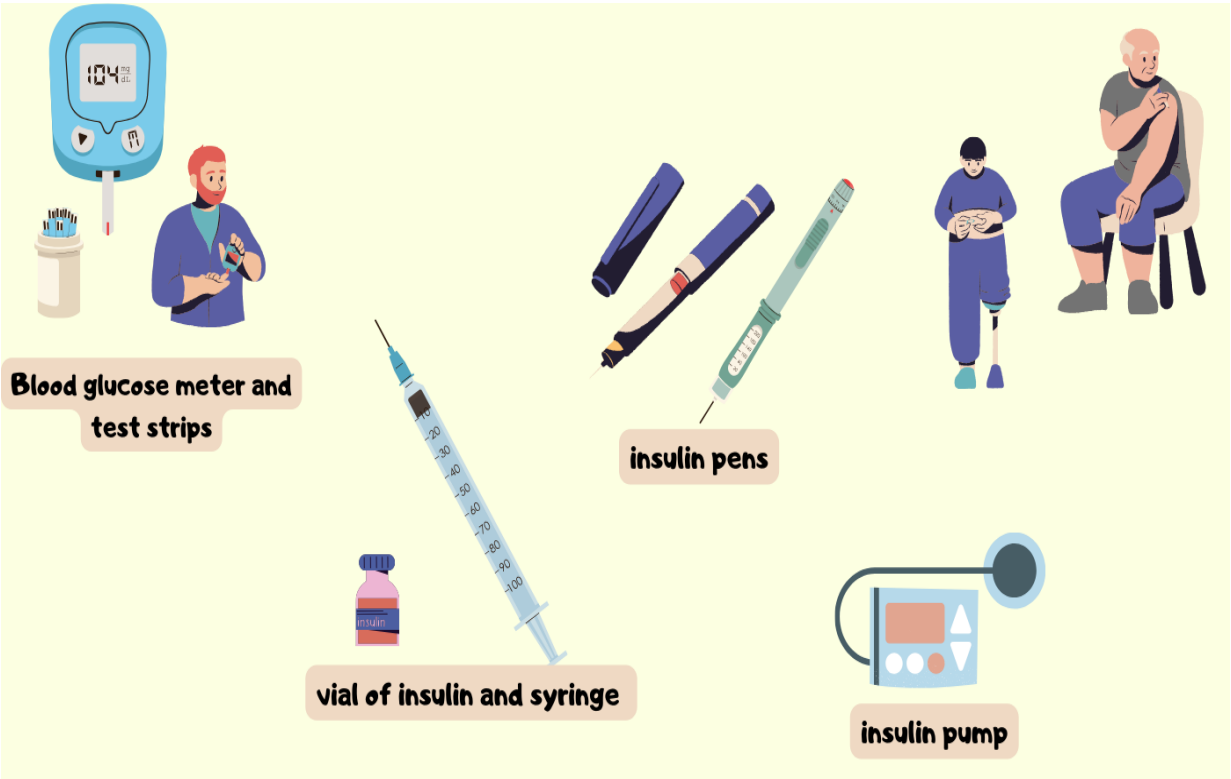


Figure 3. The different devices used for insulin injection **[48-51]**.

- **Inhaled insulin**

Inhaled insulin is an alternative for injected insulin, in this case the insulin forms a dry powder or particles, which is inhaled, the inhaled insulin is very dissolved after its inhalation and acts rapidly **[52]**.

4.2. Type 2 diabetes mellitus

Many oral drugs are employed to deal with T2DM including: Biguanides, insulin secretagogues (sulfonylureas), thiazolidinediones, DPP-4 (dipeptidyl peptidase 4) inhibitors, and SGLT2 (sodium glucose co-transporter protein 2) inhibitors **[53]**.

- **Biguanides**

Biguanides are oral antidiabetic agents that improve insulin efficiency, particularly in the muscles and liver. Metformin is the most widely used drug in this class. It acts by inhibiting the gluconeogenesis in the liver, it penetrates liver cells and attacks complex 1 of the respiratory chain in mitochondria, leading to an increase in cAMP levels and a decrease in ATP levels, activating AMPK (AMP-activated protein kinase), this activation provokes glycogen synthesis and increases insulin sensitivity **[53-58]**.

- **Sulfonylureas**

Sulfonylureas are used to stimulate insulin production by pancreatic cells. Glipizide, is widely used drug in this class, it interacts with the membrane of β-cells due to the presence of a specific receptors, which leads to the depolarization of the membrane due to the inhibition of the ATP sensitive potassium channels, then the level of Ca^{2+} augments and leads to the insulin release from the insulin vesicles **(Figure 4) [58, 59]**.

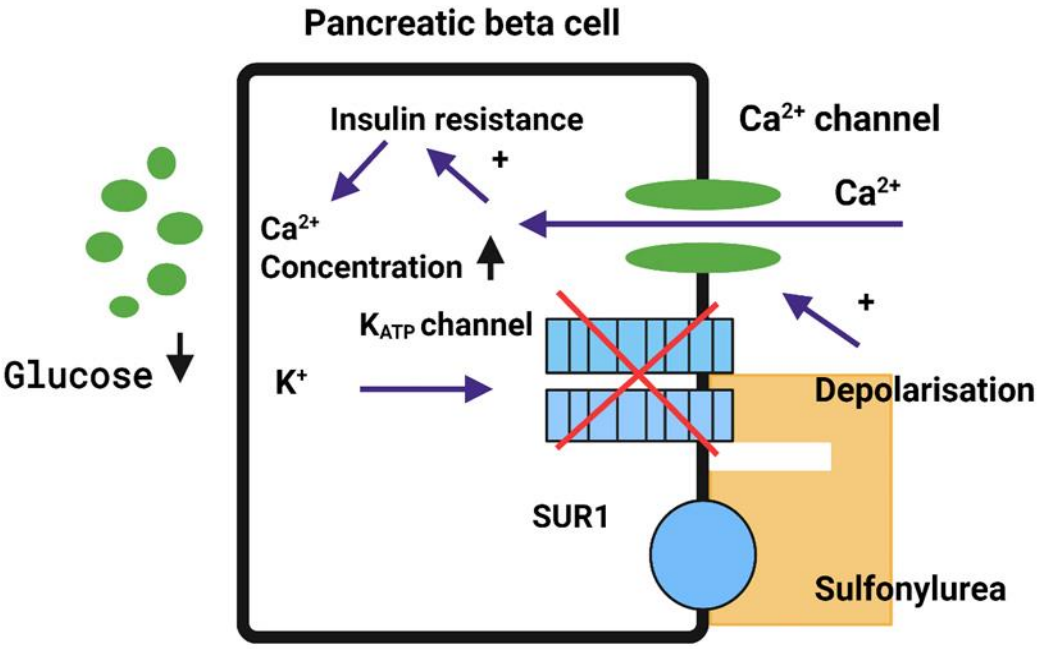


Figure 4. The mechanism of action of sulfonylureas **[60]**.

- **Thiazolidinediones**

Thiazolidinediones are a new class of oral antidiabetics that improve peripheral insulin sensitivity and reduce glycemia. Troglitazone from thiazolidinediones are an insulin sensitizing drugs because they increase the insulin sensitivity and glucose uptake in the peripheral tissues including: skeletal muscle and the liver, by effecting gene expression, they attack the nuclear receptor PPAR-γ (Peroxisome Proliferator-Activated Receptor Gamma) and activate it therefore, the expression of GLUT4 receptors and glucose metabolism increase which leads to a decrease in the level of glucose in the blood **[57, 58, 61-63]**.

- **Sodium-glucose co-transporters 2 inhibitors**

Sodium-glucose co-transporters 2 abbreviated (SGLT2) are a renal-glucose transporters, which handle the function of renal glucose reabsorption in kidneys, SGLT2, transport actively the glucose over the luminal membrane combined with Na^+ transport. Phlorizin is a widely used agent in this class as an SGLT2 inhibitor, its action leads to the blocking of intestinal glucose absorption and, as a result, glucose is excreted in the urine, helping to manage blood sugar levels **(Figure 5) [64]**.

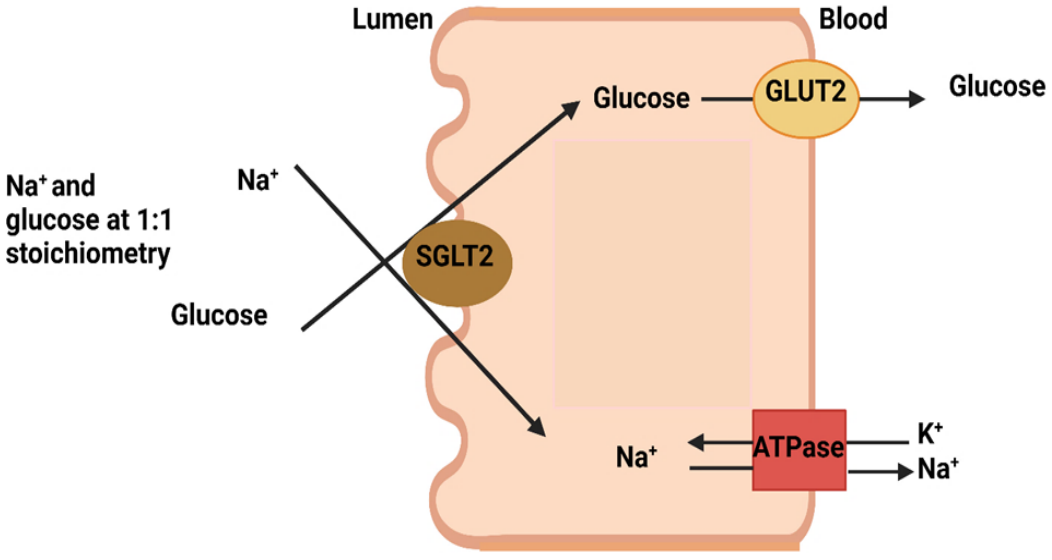


Figure 5. Mode of action of SGLT2 inhibitors **[64]**.

- **DPP-4 inhibitors**

Dipeptidyl Peptidase 4 or DPP-4 inhibitors, known as gliptins, are enzymes responsible of the inactivation of peptides such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) **[65, 66]**. The latter's are incretin hormones that are secreted by intestinal enteroendocrine L-cells and K-cells in response to food intake **[67]**. By inhibiting DPP-4 **[68]**, they prevent the rapid degradation of GLP-1 and GIP, enabling them to exert their beneficial effects on blood sugar regulation and insulin secretion **(Figure 6) [65, 66]**. The first available DPP-4 inhibitors are Sitagliptin and Vildagliptin. These compounds are orally active and have been shown to be efficacious and well tolerated **[68]**.

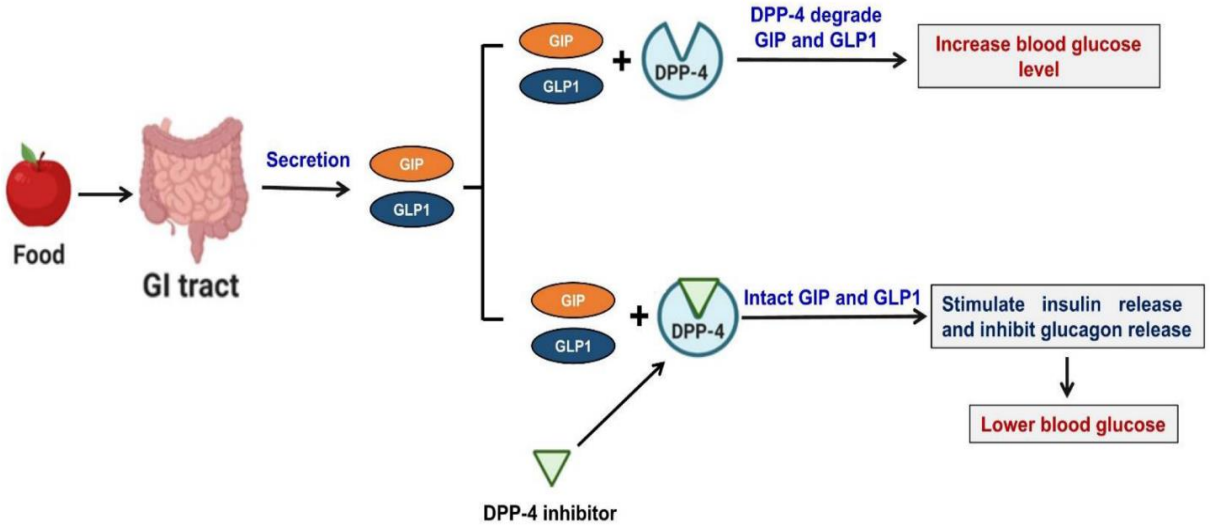


Figure 6. Mode of action of DPP-4 inhibitors **[69]**.

- **Alpha-glucosidase and α-amylase inhibitors**

Alpha-glucosidase and α-amylase are enzymes that play a crucial role in carbohydrates digestion and postprandial glycemia. Alpha-amylase is responsible for breaking down long starch chains into glucose, while α-glucosidase digests oligosaccharides and disaccharides. Inhibition of these

enzymes can slow carbohydrates digestion. Acarbose and miglitol are the drugs most commonly used as inhibitors of these two enzymes **[70, 71]**.

4.3. The artificial pancreas

Other developed technique that is used to treat diabetes mellitus, which is the artificial pancreas or also called closed loop automated insulin delivery, in this device the insulin pump (CSII) is associated with a continuous glucose monitoring (CGM) and an insulin delivery algorithm, the CGM contains a sensor and it is attached subcutaneously with the tissue and it measures the interstitial glucose level every 5-10 min, then it sends this data wirelessly to the insulin delivery algorithm which converts the signal of CGM to adjust and inject the appropriate dose of insulin **(Figure 7) [72-76]**.

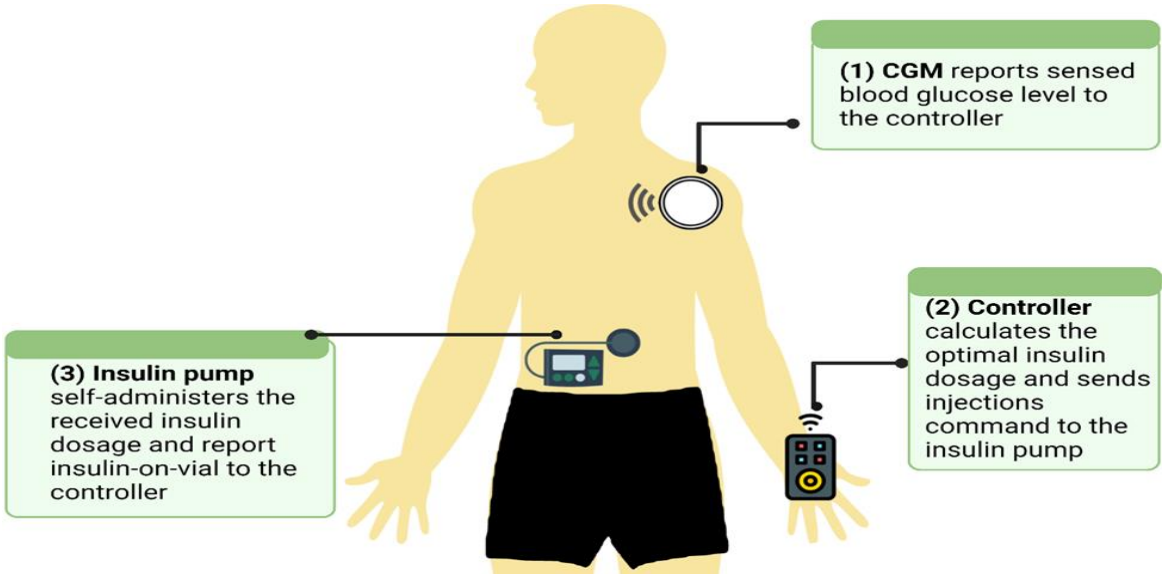


Figure 7. The artificial pancreas illustration and how it works **[77]**.

4.4. New approaches

- **Stem cell therapy**

Stem cell therapy is a new approach that could provide a cure for diabetes mellitus, as these cells have the ability to self-renew and differentiate into different cell types and, most importantly, they provide a solution to the problems of finding donors for organ transplantation **[22]**.

The stem cells form three types including:

- Adult stem cells (pancreas or bone marrow).
- Human embryonic stem cells (ESCs).
- Induced pluripotent stem cells (iPSCs) **[78-84]**.

These cells are used in *in vitro* experiments to give birth to a mature and functional pancreatic β-cells for islet transplantations, the iPSCs are obtained from adult stem cells after being genetically reprogramming *in vitro* to rise their ability to give different cell lineages, also bone marrow contains a different multipotent stem cells that could be used to generate β-cells **(Figure 8) [78-84]**.

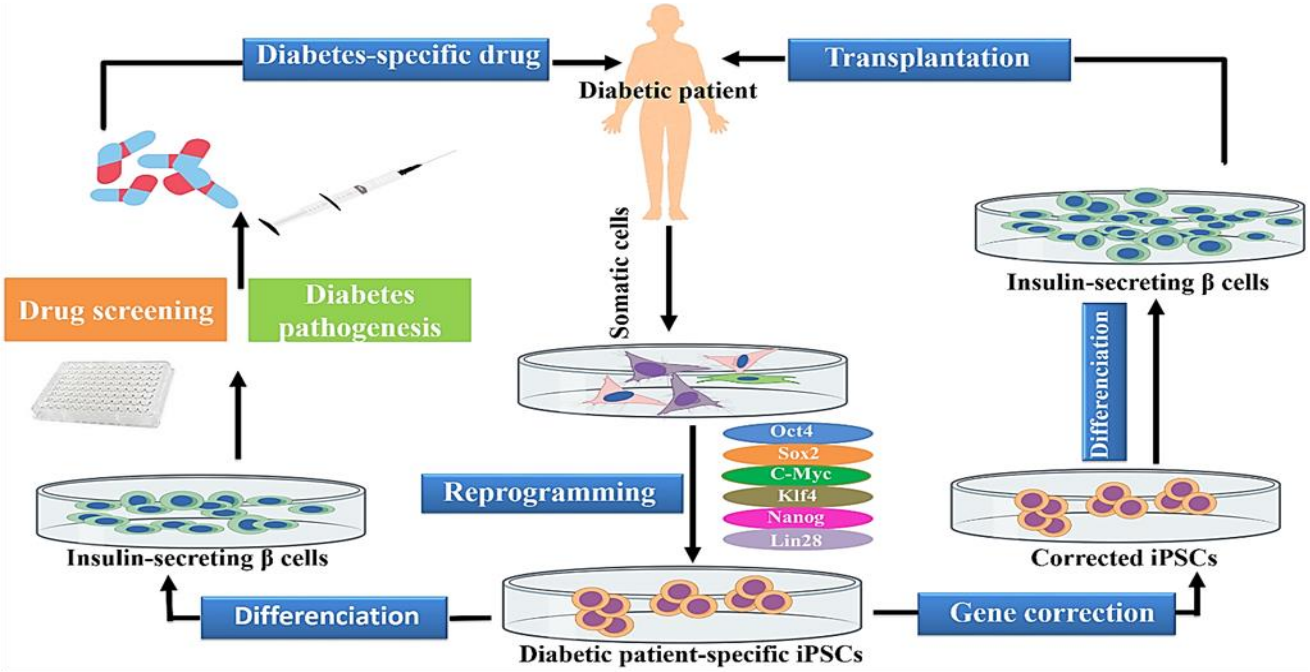


Figure 8. Stem cell therapy approach **[85]**.

- **Immunotherapy**

The auto destruction of β-cells is caused by the attack of T-cells (an autoimmune reaction), so the principle of the immunotherapy is based on the use of a specific molecules to stop this immune response, by targeting different essential elements for the autoimmune response such as:

interleukins and specific receptors for example: Teplizumab which is used as an antibody against the CD-3 of T-cells and anti-IL-1 (anakinra) **[86, 87]**.

- **Gene therapy**

Gene therapy consists of modifying the gene responsible for the development of diabetes (introducing new gene, modifying it or inactivate it), by using different vectors such as: viral vectors, this method is still under studying and tested on animals models only **[88]**.

II. Human pancreatic amylase

The pancreas is a vital organ for the human organism, secreting a variety of enzymes for food metabolism, in the form of an alkaline pancreatic juice such as proteases, lipases and especially amylases, which are responsible for the digestion of carbohydrates and our target in this study, an exocrine function of the pancreas, the secreted juice passes from the pancreas to the duodenum (small intestine), where the enzymes play their roles **[89]**.

The α-amylase is a carbohydrates digestive enzyme, that catalysis the first step of starch and polysaccharides catabolism in the small intestine and in the mouth, this enzyme is coded by two genes AMY1 and AMY2 which are located on the short arm of chromosome 1, the first one is for the salivary amylase and the second is for pancreatic amylase (HPA), the two enzymes differ by 15 amino acids with a percentage of 30%, and some of these 15 residues are catalytic amino acids, therefore they are called iso-enzymes **[90, 91]**.

HPA is a metallo-enzyme that needs the calcium ion (Ca^{2+}) and chloride ion (Cl^{-}) cofactors for its structure and activity **[7]**, its 3D crystallized structure shows that it contains 496 amino acids organized in one polypeptide chain of 56 KDa. HPA is formed by three domains: A, B and C.

- **Domain A**, is the biggest one, it starts from residue: 1 to 99 and from 169 to 404 and it forms a structure of $(\beta\text{-}\alpha)_8$ barrel fold, in this domain the active site exists which contains:

Asp197, Glu233, and Asp300, the Cl^- ion is attached by three amino acids: Arg195, Asn298, and Arg337.

- **Domain B**, is the minor, it starts from residue: 100 to 168 and it is attached to a Ca^{2+} ion to stabilize the active site via four amino acids: Arg158, Asp167, His201, and Asn100.
- **Domain C** is still under investigation to demonstrate its function **(Figure 9) [7, 90-92]**.

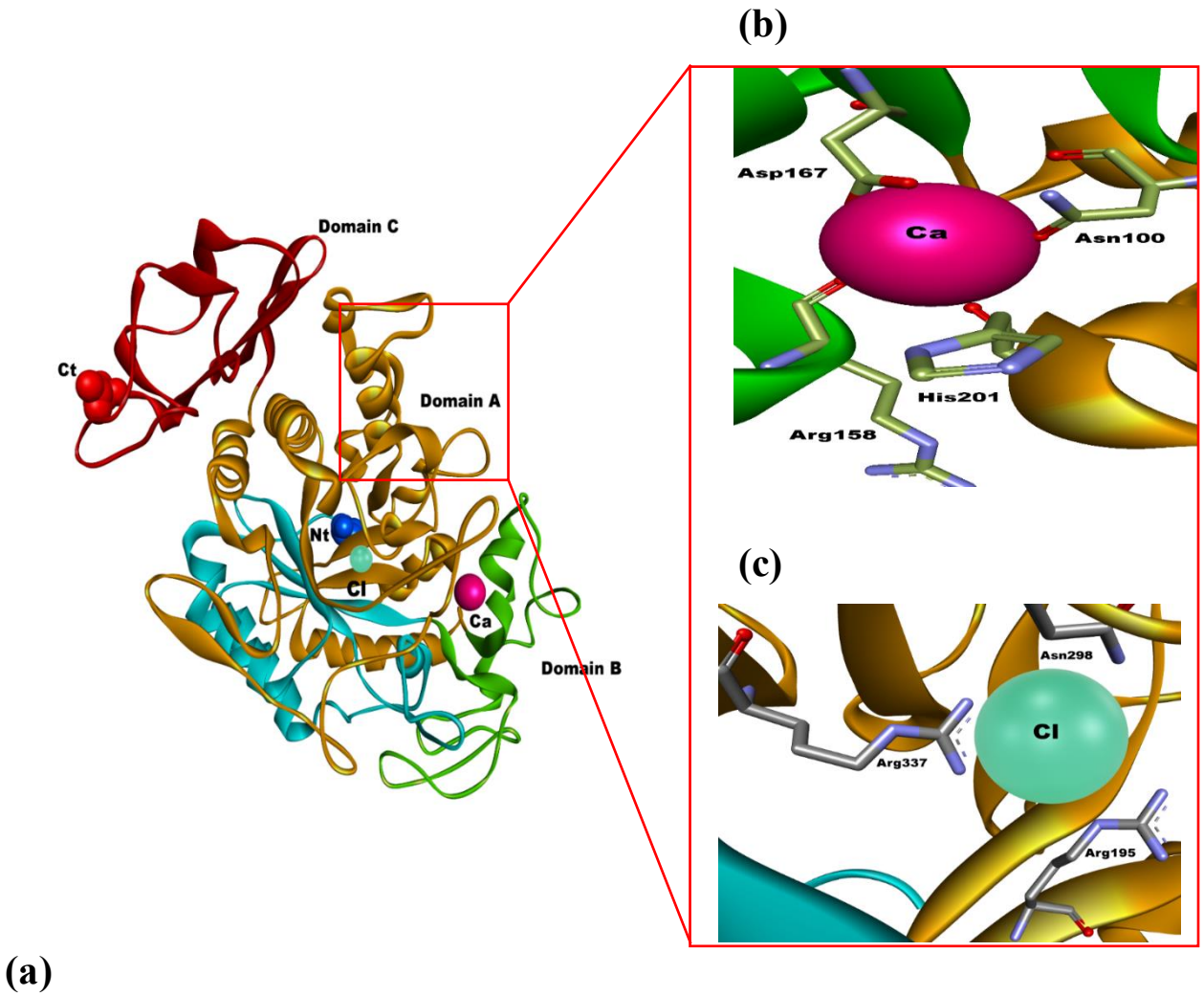


Figure 9. The 3D structure of HPA with the three domains.
(a): **Domain A**: colored in pale cyan and orange. **Domain B**: colored in green. **Domain C**: colored in red, the Nt and Ct (N and C terminal) are colored in blue and red; **(b)** is the Ca^{2+} binding site; **(c)** is the Cl^- binding site **[7]**.

The mechanism of action of HPA depends on the double displacement mode involving the catalytic triad Asp197, Asp300 and Glu233 and involves two steps:

- Asp197 (nucleophile amino acid) attacks the sugar anomeric center whereas Glu233 (acid catalyst residue) initiates the process by donating one proton to the glycosidic bond of glucose leads to the formation of a covalent intermediate.
- Then, Asp300 has a key role in the positioning of the starch within the active site **[93-96]**.

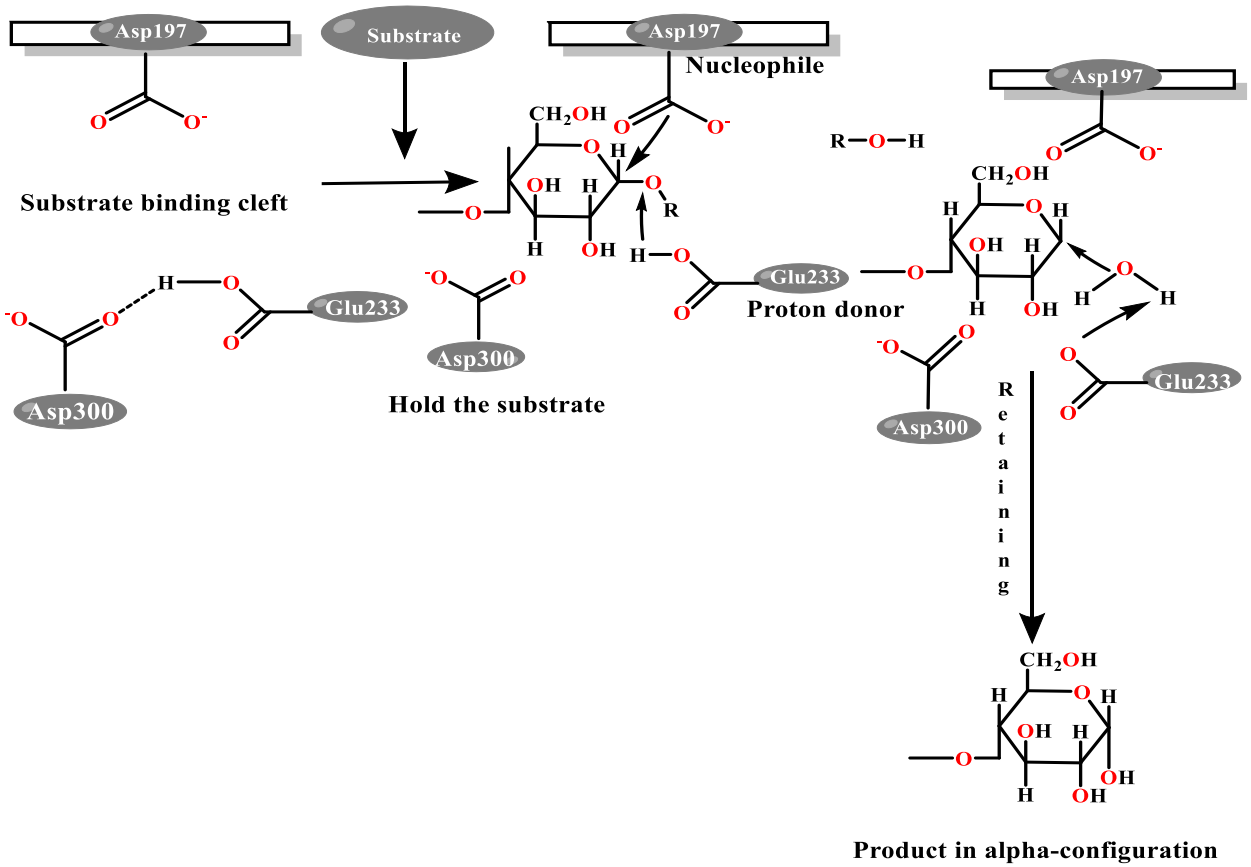


Figure 10. The double displacement mechanism of HPA for starch digestion **[93]**.

III. Studied sponges

Sponges are multicellular organisms with bodies full of pores and channels allowing water circulation, live in aquatic habitats **[97]**. They belong to the *Animalia* kingdom, and to the phylum *Porifera* meaning 'pore bearer' **[98]**, they comprise a large number of described species, these species are fixed invertebrate animals attached to rigid surfaces such as rocks, with a smooth or rough perforated body **[97]**.

They take their food through their choanocytes (collar cells) which use the filtering process of tiny organisms such as bacteria, plankton and many other small particles due to their perforated shape. Marine sponges are also a very interesting target for research, as they form a symbiotic association with various micro-organisms, resulting in a varied production of active substances **[9, 97, 99-103]**.

Marine sponges are a rich source of secondary metabolites, producing several classes of secondary metabolites to defend themselves against predators, to communicate and to compete with other animals in their environment. They have biological activities such as antimicrobial and antiparasitic, due to their symbiotic or parasitic associations with microorganisms, making them a great treasure for drug discovery **[104-106]**.

In our study, we selected four sponges such as: *Tectitethya crypta* **(Figure 11)**, *Stylissa massa* **(Figure 12)**, *Acanthella cavernosa* **(Figure 13)**, *and Agelas dispar* **(Figure 14)**, based on their potential in alkaloids. Their Linnaean taxonomy and geographical distributions were obtained on April 15, 2024 from (https://www.marinespecies.org and https://spongeguide.uncw.edu) and the guide: Splendid sponges of Palau **[103]**.

1. *Tectitethya crypta*

Common name: Volcano sponge **[107]**.

1.1. Taxonomy of the sponge

Kingdom:	***Animalia***
Phylum:	*Porifera*
Class:	*Demospongiae*
Order:	*Tethyida*
Family:	*Tethyidae*
Genus:	*Tectitethya*
Species:	*Tectitethya crypta*

1.2. Geographical distribution and morphology

Tectitethya crypta is a widely common in the region of : Long Key and in Marathon and also in Bahamas but with different number of species in these places **[108, 109]**. *Tectitethya crypta* has a hemispherical to conical shape it means globular, with a thick masse, covered with a layer of sediment and it is full of oscules with a papillated surface (irregular surface), this sponge has a unique color, colored by the is black with a little bit of green which is a dark olive green color **[108, 109]**.

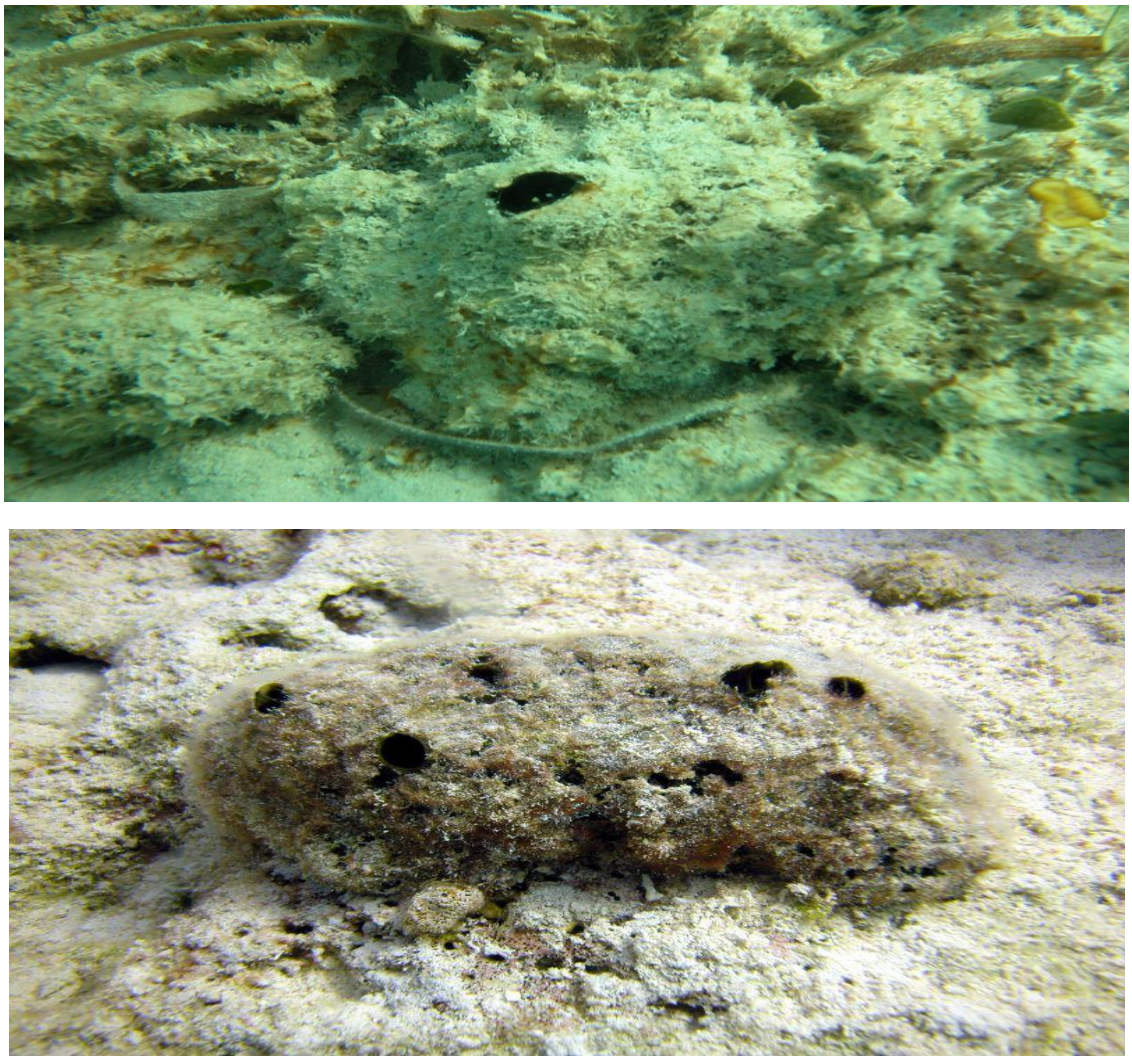


Figure 11. Photos represent: *Tectitethya crypta* (Obtained on April 15, 2024), from the sponge guide webserver https://spongeguide.uncw.edu **[108]**.

2. *Stylissa massa*

Common name: Mango sponge **[110]**.

2.1. Taxonomy of the sponge

Kingdom :	***Animalia***
Phylum	*Porifera*
Class :	*Demospongiae*
Order :	*Scopalinida*
Family :	*Scopalinidae*
Genus :	*Stylissa*
Species :	*Stylissa massa*

2.2. Geographical distribution and morphology

Stylissa massa is distributed in the Indo pacific **[111]**. It can have a single or multilobed structure with a thick sheet or columns and has a 75 cm high and 3-20 cm broad with oscules of 5-10 mm in diameter. It also has a compressible texture with an orange fluorescent tint **[103]**.

Figure 12. Photos represent: *Stylissa massa* (Obtained on April 15, 2024 from the guide: Splendid sponges of Palau) **[103]**.

3. *Acanthella cavernosa*

Common name: Orange lace sponge **[112].**

3.1. Taxonomy of the sponge

Kingdom :	***Animalia***
Phylum	*Porifera*
Class :	*Demospongiae*
Order :	*Bubarida*
Family :	*Dictyonellidae*
Genus :	*Acanthella*
Species :	*Acanthella cavernosa*

3.2. Geographical distribution and morphology

Acanthella cavernosa is distributed in the west central pacific, northwest Australia, and southest Asia **[103]**. It has a tiny spherical shape, up to 20 cm high and from 8-10 cm thick with oscules of 3-5 mm diameter, it has a compressible texture and it is difficult to rip, it is colored in dark orange **[103]**.

Figure 13. Photos represent: *Acanthella cavernosa* (Obtained on April 15, 2024 from the guide: Splendid sponges of Palau) **[103]**.

4. *Agelas dispar*

Common name: Brown encrusting sponge **[113].**

4.1. Taxonomy of the sponge

Kingdom :	***Animalia***
Phylum	*Porifera*
Class :	*Demospongiae*
Order :	*Agelasida*
Family :	*Agelasidae*
Genus :	*Agelas*
Species :	*Agelas dispar*

4.2. Geographical distribution and morphology

The genus of *Agelas* has a large geographical distribution in tropical and subtropical oceans, like Okinawa sea, Caribbean sea, and south China sea **[114]**. *Agelas dispar* has a gigantic round shape with a dark brown tint. It has little circular oscules and has a height of 10-40 cm and a diameter of 20-50 cm **[115]**.

(a)

(b)

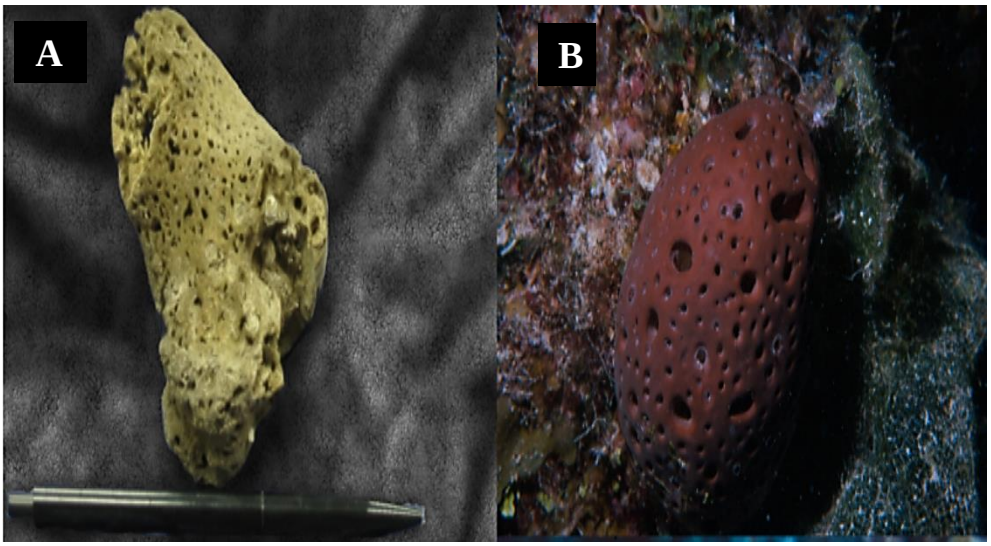


Figure 14. Photos represent: *Agelas dispar* (Obtained on April 15, 2024 from the sponge guide webserver https://spongeguide.uncw.edu **[108]**; **(b): A:** The holotype of *Agelas dispar*; **B:** Represents the massive specimen of *Agelas dispar* from Bahamas **[115]**.

MATERIAL AND METHODS

I. Software's and programs

1. Discovery studio visualizer

Discovery studio visualizer (DSV) is an open-source software package used to design and simulate small molecule and macromolecule systems such as ligands conceptions, viewing and editing 3D structure of proteins. It is used in pharmacophore modeling, drug design **[116, 117]**, and in the docking results analyzing by showing ligand-receptor interactions **[118-120]**.

2. AutoDockTools 1.5.7

AutoDockTools (ADT) is an open-source program used to prepare receptors for molecular docking by creating the grid box in coordinate form (x, y and z) and generating the PDBQT file format required for AutoDock Vina **[121-124]**.

3. AutoDock Vina

AutoDock Vina (ADV) is a user-friendly open-source program for molecular docking and virtual screening developed by the Scripps Research Institute. It predicts affinity and receptor-ligand interactions based on binding energy in kcal/mol **[125-128]**.

4. ChemDraw professional

ChemDraw professional is a user-friendly open-source program used to:

- Draw and generate 2D chemical structures for our study by name or IUPAC name, and calculate their physico-chemical properties.
- Generate chemical reactions and calculate their stoichiometry.
- Generate intelligent biochemical arts.
- Predict nuclear magnetic resonance (NMR) spectra for compounds, and providing a multi-file format choice for results export **[128-132]**.

II. Databases and web servers

1. Protein Data Bank

Protein Data Bank (PDB) is the first database in biological sciences, managed by the protein data bank organization **[133]**. PDB is the single universally gallery of a large number of 3D structures of macromolecules such as proteins, DNA, RNA, and their associated molecules (ligand, substrate, and cofactors) **[134, 135]**.

2. PubChem

PubChem is a publicly accessible database at the National Center for Biotechnology Information (NCBI), organized into different data collections such as bioassays, compounds and substances. PubChem is used in many research fields, including clinical biochemistry, medicine and food sciences. It provides information on the different structures of molecules, e.g. lipids, carbohydrates, alkaloids and synthetic compounds, and can generate them in 2D and 3D structures, or provide their literatures **[136-138]**.

3. Way2Drug

Way2Drug is an online server provides a comprehensive computational tool that enables researchers and drug developers to access information about approved drugs and predict the potential biological properties of drug candidates, supporting the drug discovery and development process **[139].**

4. PreADMET v2.0

PreADMET is a user-friendly interface online server used to access and analyze various properties of chemical compounds and predict rapidly the absorption, distribution, metabolism, excretion, toxicity parameters abbreviated ADMET of a compound, it means its pharmacokinetic profile by using molecular descriptors, *in vivo*, and *in vitro* models supporting the early stages of drug discovery and development **[140-144]**.

5. LigRMSD 1.0

LigRMSD is an open-source web server for automatic matching and RMSD calculations of identical or comparable chemical substances. The LigRMSD server includes several scripts developed for user interaction, data organization, manipulation and visualization. The web server interface is highly intuitive and enables the user to obtain the RMSD in two cases: between two molecules or in relation to several references **[145]**.

III. PASS study

Prediction of activity spectra for substances or PASS is a free online server powered by the Institute of Biomedical Chemistry (IBMC) **[146-149]**. PASS offers 250,407 recognized biologically active compounds **[150]**. We used it for the prediction of biological activities of our molecules toward the HPA using Way2Drug webserver (https://www.way2drug.com/index.php). The different biological activities are predicted based on their 2D structure using multilevel neighbors of atoms (MNA) method, then the choice of the alkaloids was performed based on their Pa/Pi values (Pa with a value over 0.3 is selected), the results are presented as Pa (probability for active compound) and Pi (probability for inactive compound) **[147, 148, 150-152]**.

IV. Pharmacokinetic features-ADMT

Pharmacokinetics (PK) is the prediction of ADMT properties, ADMT standing for absorption, distribution, metabolism and toxicity **[153, 154]**, ADMT study plays a vital role in drug design and discovery, as these parameters are very important for the prediction of drug safety and quality **[155-158]**. To verify the pharmacokinetic characteristics of our compounds, we used the webserver preADMET v2.0 **[159]** and the control drug, Acarbose.

PreADMET v2.0 offers a user-friendly interface and improved prediction accuracy from earlier *in vitro* and *in vivo* tests that were gathered from various databases. Comprehensive access to small

molecules, clinical trials, bioassays, chemical registration, and analytical methods including mass spectrometry (MS) and NMR **[160]**. The used ADMT parameters are:

- **The caco-2 cell permeability (nm/sec)**: originated from colon carcinoma: It is an *in vitro* test used to predict the passage of a drug through the intestinal epithelium because these cells share multiple morphological similarities with normal enterocytes **[161-163]**. So caco-2 cell is used to detect the drugs absorption because they mimic the intestine epithelial barrier.
- **Human intestinal absorption HIA (%)**: Is an essential parameter for determining the bioavailability of a drug when taken orally, and the drug cannot reach the systemic circulation without being absorbed **[164, 165]**.
- **Blood brain barrier (BBB)**: Is a safeguarding semi-permeable membrane, composed of endothelial cells **[166]**, located between the blood and the brain, which protects the central nervous system from harmful substances with a molecular weight over 500 KDa **[167, 168]**.
- **P-glycoprotein (P-gp)**: Is an ATP binding cassette transmembrane transporters, located in the liver, kidneys, small intestine and brain, its function is to eliminate harmful substances from cells **[169, 170]**.
- **Plasma protein binding (%)**: Is the measurement of a non-covalent interaction between plasma proteins such as: albumin and globulins and endogenous or exogenous compounds like: drugs all along the blood circulation to the target cells, it influences pharmacokinetics and pharmacodynamics of drugs **[171, 172]**.
- **Cytochromes P450 (CYPs)**: Are a family of membrane bond hemoproteins, expressed principally in the liver and intestine also found in: heart, brain, and lungs, these enzymes are involved in drugs and endogenous molecules metabolism **[173-175]**.

- **The MDCK (Madin-Darby Canine Kidney) cell permeability (nm/sec)**: Are a normal kidney cells of a cocker spaniel dog used to predict the absorption of drugs (from the intestine to the blood circulation) **[176, 177]**.

The toxicity of our compounds was predicted with Ames test which is a simple test used to evaluate the mutagenicity potential **[178]**. Furthermore, mice and rats were used as model organisms to predict carcinogenicity due of their genetic and physiological similarities to humans. Our compounds have been checked for their potential to inhibit the human ether-related gene (HERG) channel, in order to guarantee cardiac safety **[179]**.

V. Molecular docking

Molecular docking (MD) is a computational simulation procedure models the best orientations and conformations of the ligand on a protein surface (also known as the receptor or target), and scores the ability of these two objects to bind and interact together **[180]**. MD involves simulating non-covalent interactions between ligand-protein complex, in order to predict the conformation and their binding affinity. We performed MD to investigate the main function of the HPA with five alkaloids like: ALK1 (Spongothymidine), ALK2 (Spongouridine), ALK3 (Spongiacidin C), ALK4 (Deoxynebularine), and ALK5 (Pyridinebetaine A) as possible inhibitors to identify the inhibition mechanism and the involved interactions. The used compounds were retrieved from PubChem compound database **[181]** in SDF format. Then saved in PDB format using DSV. Subsequently, the PDB files were converted to PDBQT form using ADT program to prepare the ligands for molecular docking. Until now, there are no previous studies of the antidiabetic effects of the selected compounds. Therefore, this study is original.

Initially, the crystal structure of the alpha amylase (PDB ID: 3BAJ) was obtained from the PDB, which represents the HPA; it contains a single polypeptide chain named (A) with 496 amino acids, and three heteroatoms: 2-acetamido-2-deoxy-beta-D-glucopyranose (NAG), nitrate ion ($NO3^-$) and

calcium ion (Ca^{2+}), it was found complexed with acarbose ligand, this latter was set as positive control for docking validation of this target and the root mean square deviation (RMSD) between the two superimposed molecules was calculated. The system was validated in order to increase the ligand enrichment, which is required in the MD process.

The MD was set as a specific docking type given the full flexibility to the inhibitor and catalytic site flexibility of the receptor. The preparation of the protein goes through four steps:

- **The first step (1)** needs to remove unnecessary substances including; water molecules, heteroatoms, ligands and co-ligands, except the required in the active site.
- **The second step (2)** consists adding polar hydrogens and partial charges to the enzyme structure, this was done using ADT program.
- **The third step (3)** handling the setting of size and center of the grid box (GB) 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).
- **The fourth step (4)** after defining the GB, we have performed the MD using the ADV.

After the realization of molecular docking the calculation of Ki (the inhibition constant) is necessary to improve the affinity of our target the human pancreatic amylase for the selected alkaloids by the application of the following equation:

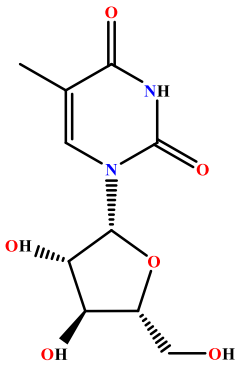
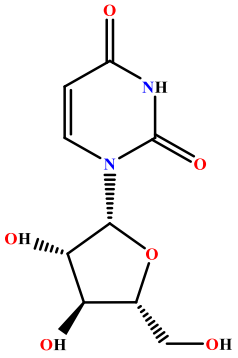
$$ki = \mathrm{e}\,\frac{\text{Binding energy}\times 10^{3}}{1.986 * 298}\times 10^{6}$$

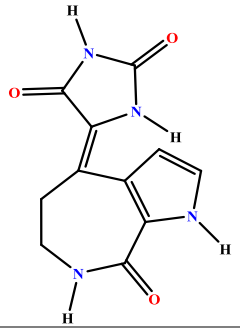
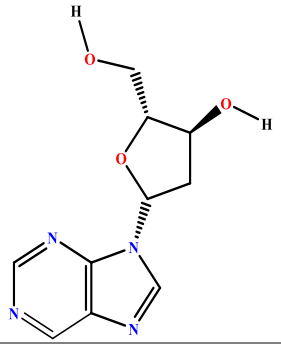
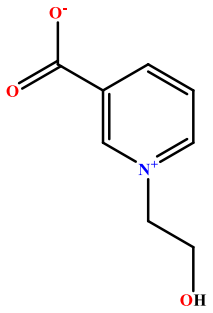
RESULTS AND DISCUSSION

I. PASS study

In this study, the five selected alkaloids are chosen based on their predicted HPA inhibition activity. The results obtained are presented in table 1. The primary PASS results indicate moderate Predicted Biological Activity Numbers (PBAn) for spongothymidine (ALK1), spongouridine (ALK2), deoxynebularine (ALK4) and pyridinebetaine A (ALK5), ranging from 982 to 1908, and a low PBAn for spongiacidin C (ALK3), with a value of 658. Although ALK5 achieved the highest PBAn value of 1908, indicating its diverse biological activities. The spongiacidin C (ALK3) presents the best inhibitor among the other alkaloids because it has the best Pa/Pi values (**Table 1**).

Table 1. The predicted biological activity for the five chosen compounds with their 2D structures, Pub ID, molecular weight and chemical formula.

Compound	2D structure	Source	Alpha-amylase inhibitory activity from PASS	Reference
ALK1 (Spongothymidine) Pub ID: 65049 MW: 258.23 g/mol F: $C_{10}H_{14}N_2O_6$		***Tectitethya crypta***	**Pa: 0.361** **Pi: 0.021**	**[100, 182]**
ALK2 (Spongouridine) Pub ID: 18323 MW: 244.2 g/mol F: $C_9H_{12}N_2O_6$			**Pa: 0.404** **Pi: 0.015**	

ALK3 **(Spongiacidin C)** Pub ID: 10634145 MW: 246.22 g/mol F: $C_{11}H_{10}N_4O_3$		***Stylissa massa***	**Pa: 0.432** **Pi: 0.010**	**[183]**
ALK4 **(Deoxynebularine)** Pub ID: 65148 MW: 236.23 g/mol F: $C_{10}H_{12}N_4O_3$		***Acanthella cavernosa***	**Pa: 0.337** **Pi: 0.024**	**[184]**
ALK5 **(Pyridinebetaine A)** Pub ID: 104238 MW: 167.16 g/mol F: $C_8H_9NO_3$		***Agelas dispar***	**Pa: 0.434** **Pi: 0.012**	**[114, 185]**

Pub ID: PubChem ID **MW:** Molecule Weight **ALK:** Alkaloid **F:** Molecular formula
Pa: Probability for active compound **Pi:** Probability for inactive compound

II. ADMT study

The ADMT parameters predicted for all alkaloids are presented in table 2. The results indicate low Caco-2 cell permeability, ranging from 0.76 nm/sec to 4.63 and 8.87 nm/sec, for ALK4, ALK1 and ALK2 respectively suggesting difficulties in crossing this restrictive membrane. However, ALK3 and ALK5 indicate moderate permeability. Additionally, the alkaloids exhibit different water solubility. In addition, ALK4 and ALK5 demonstrate high human intestinal absorption, ranging from 85.73 to 88.10 %, except for ALK1, ALK2 and ALK3, the latter has a low absorption value

of 35.28% (ALK2) (**Table 2**). These results indicate favorable absorption characteristics for the majority of the alkaloids. Moreover, P-glycoprotein inhibition was not found in all alkaloids that aid in pumping these substances out of cells when they become toxic **[186]**.

Table 2. ADMT parameters of the five selected alkaloids.

Pharmacokinetics	ALK1	ALK2	ALK3	ALK4	ALK5	Control
Absorption						
Caco-2 cell permeability (nm/sec) > 20	4.63	8.87	21.10	0.76	20.78	9.44
Human intestinal absorption **(HIA %) 80 to 100 %**	38.22	35.28	56.04	85.73	88.10	0.00
Water solubility (mg/l)	2817.46	30735.6	4097.25	45993.3	40093.4	5793.5
P-glycoprotein inhibition **A substrate of it indicates high levels absorption**	Non	Non	Non	Non	Non	Non
Distribution						
Blood-brain barrier penetration (C.brain/C.blood) **> 2 cross the blood–brain barrier easily**	0.25	0.36	0.11	0.10	0.09	0.02
MDCK cell permeability (nm/sec) **Low (< 1 nm/s), moderate (1-10 nm/s), and high (>10 nm/s)**	0.52	0.52	1.42	0.66	0.56	0.51
Plasma protein binding (%) **80 to 100 % is considered high, 50 to 80 % (moderate), < 50 % (low)**	9.34	8.08	8.88	17.14	0.00	0.00
Skin permeability (logKp, cm/hour) **< -2.5 considered high permeable**	-5.12	-5.15	-5.12	-4.98	-4.31	-5.17
Metabolism						
Cytochrome P450 2C19 inhibition	Non	Non	Non	Inhibitor	Inhibitor	Non
Cytochrome P450 2C9 inhibition	Non	Non	Non	Inhibitor	Inhibitor	Non
Cytochrome P450 2D6 inhibition	Non	Non	Non	Non	Non	Inhibitor
Cytochrome P450 2D6 substrate	Non	Non	Non	Non	Non	Weakly
Cytochrome P450 3A4 inhibition	Inhibitor	Inhibitor	Non	Inhibitor	Non	Inhibitor
Cytochrome P450 3A4 substrate	Weakly	Weakly	Substrate	Weakly	Non	Substrate
Toxicity						
Ames test	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Non mutagen
Carcinogenicity (Mouse)	Negative	Negative	Negative	Negative	Negative	Negative
Carcinogenicity (Rat)	Negative	Negative	Negative	Negative	Negative	Negative
HERG_inhibition	Low risk	Low risk	Medium risk	Medium risk	Medium risk	Ambiguous

HERG**: Human ether related gene channel,** MDCK**: Mandin Darby Canine Kidney,** Caco-2**: Human colorectal carcinoma,** Kp**: skin permeability constant**

The distribution profile of our predicted alkaloids indicates challenges in crossing the blood-brain barrier (BBB), with scores ranging from 0.09 to 0.36. These scores are comparatively high when compared to the standard values (0.02, indicating a difficult crossing of the BBB). Consequently, their potential neurological effects are diminished due to the low likelihood of crossing this barrier. However, all the alkaloids exhibit the ability to pass through the skin easily.

All compounds exhibited a consistently low percentage of plasma protein binding, indicating weak binding to plasma proteins such as albumin and globulins. This suggests that our alkaloids have a weak interaction with these proteins, which in turn requires lower doses to reach their meant targets. Before the molecules are released again, the binding fraction of the alkaloids should not saturate all of the albumin's binding sites.

Metabolic profile analysis revealed that ALK4 and ALK5 inhibited CYP 2C19 and CYP 2C9, while the other compounds were not inhibitors for these enzymes. Thus, no alkaloids inhibited CYP 2D6. ALK1, ALK2, and ALK4 were inhibitors for CYP3A4; the other alkaloids were non-inhibitor, weakly inhibitors, or substrates. Xenobiotics that are rapidly metabolized may not remain in the bloodstream long enough to produce the desired effects. Conversely, if their metabolism is slow, they may accumulate and cause toxicity, even if they show promise.

The toxicity profile indicates consistent outcomes in terms of mutagenicity in the Ames tests (as shown in table 2). The majority of the compounds displayed negative results for mouse and also for rat carcinogenicity, which is more closely related to human genes, indicating a low potential for carcinogenicity. The inhibition of human ether-related gene channel posed a low risk for both of ALK1 and ALK2 and medium risk for ALK3, ALK4, and ALK5. Consequently, it is important to adjust drug doses, administration routes, and treatment durations to address certain weak pharmacokinetic parameters.

III. Molecular docking

Depending on the minimum energy values (kcal/mol), ki (µM), and the RR percentage: ALK1, ALK3, and ALK4 were the best inhibitors among the other tested alkaloids **(Table 3)**. ALK2 was reported as moderate inhibitor. However, ALK5 was the weakest compared to the others. ALK3 was the closest to the control and was considered a possible inhibitor model for HPA with the best-saved energy. Otherwise, all alkaloids save RR percentage of 100%. The results are summarized in (**Table 3)**.

Table 3. The results of molecular docking: catalytic amino acids are colored in red.

Molecule	ΔG Affinity (kcal/mol)	Ki (µM)	RR %	Closest residues	Hydrogen bonds / Length (Å)	Hydrophobic interactions
Acarbose (Control)	-9.0	0.24	100	TRP59, GLN63, THR163, ARG195, ASP197, HIS201, GLU233, HIS299, ASP300, HIS305	ARG195 (2.15) GLU233 (3.08, 2.79) ASP300 (2.05, 2.62, 2.70) HIS299 (2.73) THR163 (3.00) TRP59 (2.57) ASP197 (3.09, 2.13) GLN63 (2.74) HIS201 (2.34) HIS305 (2.99, 3.07)	-
ALK1 (Spongothymidine)	-6.6	14.34	100	TYR62, THR163, LEU165, ASP300, HIS305	THR163 (2.54) HIS305 (2.77) ASP300 (3.05, 2.14) TYR62 (2.52)	Alkyl Pi-Alkyl
ALK2 (Spongouridine)	-6.1	33.40	100	TYR62, THR163, ASP197	ASP197 (2.54) THR163 (2.16)	Pi-Pi Stacked
ALK3 (Spongiacidin C)	-7.5	2.88	100	TRP59, TYR62, LEU165, ASP197, HIS305	ASP197 (2.01)	Pi-Sigma Pi-Pi Stacked Alkyl
ALK4 (Deoxynebularine)	-6.4	20.11	100	TYR62, THR163, LEU165, ASP197, GLU233, ASP300	ASP300 (2.45) ASP197 (2.56, 2.86) THR163 (2.93)	Pi-Sigma Pi-Alkyl
ALK5 (Pyridinebetaine A)	-5.4	109	100	TYR62, ASP197, GLU233	ASP197 (2.51, 2.38) GLU233 (2.51)	Pi-Alkyl

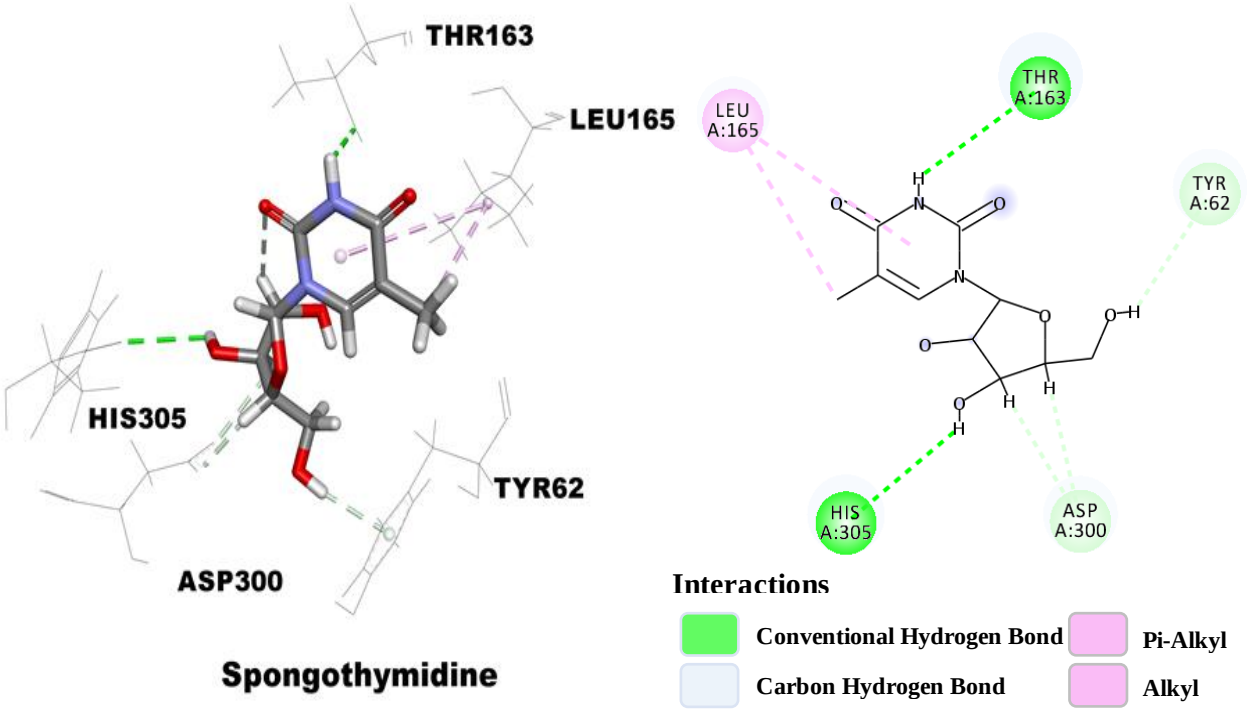
RR: repetition ratio, **Å**: angstrom

Alpha-Amylase or 1,4-α-D-glucan-glucanohydrolase (EC 3.2.1.1) is an endoenzyme of the hydrolases family, it is synthesized both in the salivary glands and in the rough endoplasmic reticulum of the pancreas, it plays an important role in the first stage of the digestion of starch and polysaccharides such as glycogen. The α-amylase dysregulation leads to carbohydrates abnormal absorption particularly in relation to the diabetes **[92]**.

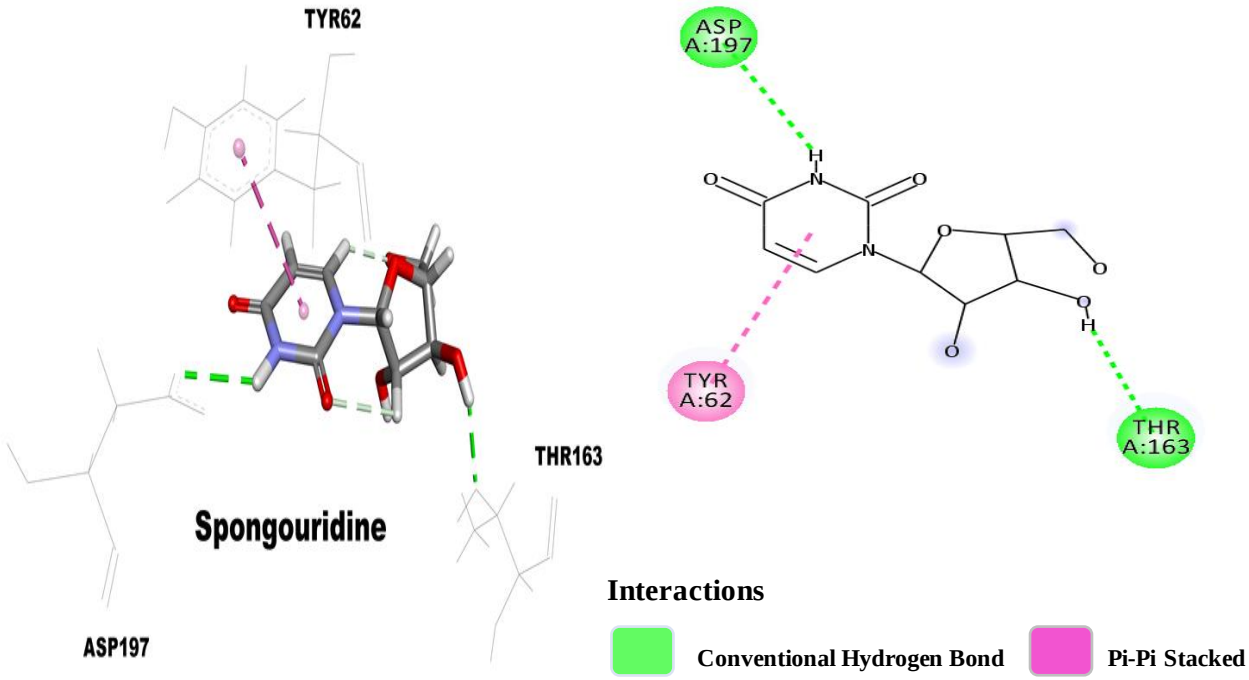
Its crystal structure was found complexed with acarbose as mentioned previously. The docking validation shows that acarbose was bound in the active site and formed mostly hydrogen bonds with several residues, including the important: Asp197, Glu233, Asp300, His299, Trp59, Gln63, Thr163, and His201. Moreover, no hydrophobic interactions were detected.

ALK1 and **ALK2** are nucleoside alkaloids **[100, 182]** that differ by the presence of the methyl group in favor of ALK1, while **ALK4** is a purine deoxyribonucleoside alkaloid **[184]**. They exhibited similar binding energy (**Table 3**). **ALK4** ranked third superior to ALK2, which ranked fourth, among them; **ALK1** performed best with the second rank based on energy value. The pyrimidine ring of ALK1 was highly reactive, enabling it to incorporate into the HPA active site and establish important hydrogen bonds with Tyr62, Thr163 and Asp300. These residues generally interact in the following way: Tyr62 interacts with the hydroxyl group of the ribose ring, Thr163 interacts with the amine function of the pyrimidine ring and Asp300 interacts with the two hydrogen atoms of the C3 and C4 of the ribose ring. ALK1 also engages in hydrophobic π-alkyl and alkyl interactions with Leu165 **(Figure 15)**. Cyclization of the pyrimidine ring in ALK1 provided a structural advantage, increasing its affinity for HPA compared with ALK4, which contains a purine ring with fewer interactions saved (**Figure 15**). In contrast, **ALK2** has no methyl group attached to the pyrimidine ring, which remarkably influenced its binding to HPA, confirming the importance of methyl groups in preserving hydrophobic interactions and participating in overall stability.

(1) A (2)


THR163
LEU165
HIS305
TYR62
ASP300
Spongothymidine
THR A:163
LEU A:165
TYR A:62
HIS A:305
ASP A:300
Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Carbon Hydrogen Bond
Alkyl

B


TYR62
THR163
ASP197
Spongouridine
ASP A:197
TYR A:62
THR A:163
Interactions
Conventional Hydrogen Bond
Pi-Pi Stacked

C

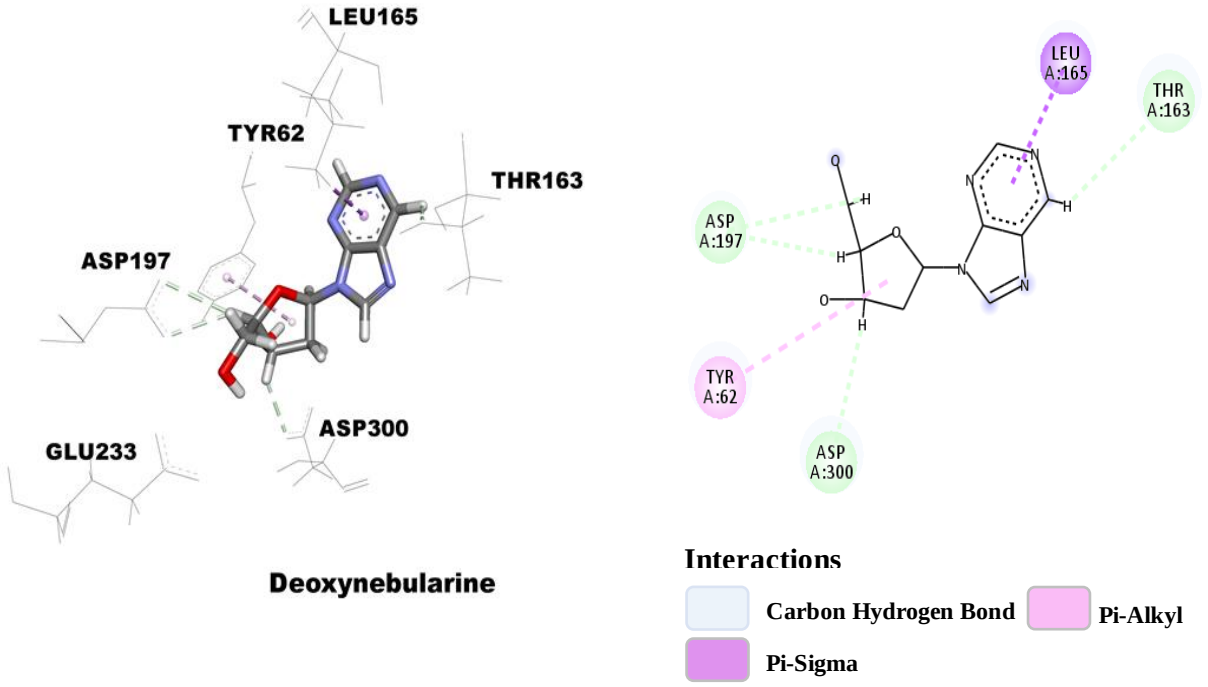


Figure 15. The 3D (1) and 2D (2) structures of the best pose after docking of ALK1 (A), ALK2 (B), and ALK4 (C) displayed in stick form and colored using default atom colors.

ALK3 ranked first (**Figure 16**), a pyrrole alkaloid **[183]**, composed of three attached heterocycles: a pyrrole, an azepine and an imidazolidine. It has the best binding energy compared with other HPA-targeting compounds (**Table 3**), which explains its high stability and suggests that it could serve as a perfect model for HPA. It bonds by a crucial conventional hydrogen bond with Asp197, and by four types of hydrophobic interactions: π-π stacked, π-sigma, and alkyl, with Tyr62, Trp59 and His305, safeguarded mainly by the imidazolidine heterocycle moiety. The latter was the most important as it preserved most of the hydrophobic interactions and participated in the stability of ALK3 inside the HPA (**Figure 16**).

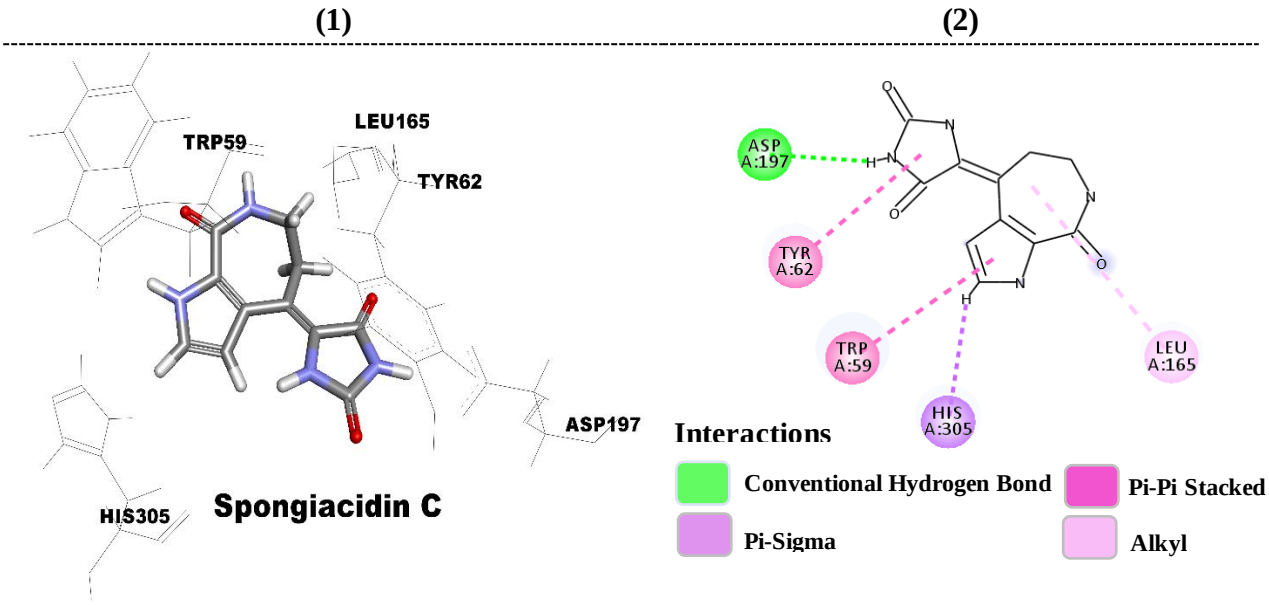


Figure 16. The 3D (**1**) and 2D (**2**) structures of the best pose after docking of ALK3 displayed in stick form and colored using default atom colors.

ALK5 ranked last among HPA-targeting compounds, it is a betaine alkaloid **[114]** that contains a pyridinium ring attached to both a carboxyl and an alcohol function, it exhibits low binding energy (**Table 3**) compared to other HPA-targeting compounds with minimal saved interactions, in addition to its low molecular weight compared to other compounds, which explains its reduced stability and suggests that it may not serve as a perfect model for HPA. ALK5 binds to HPA via two conventional hydrogen bonds with Asp197 and Glu233 with its alcohol tail, as well as a hydrophobic π-alkyl interaction, with Tyr62 with its pyridinium ring (**Figure 17**). Ultimately, the bonds/interactions saved were not sufficient to achieve the perfect pose, therefore, betaine alkaloids could not be drug candidates for HPA.

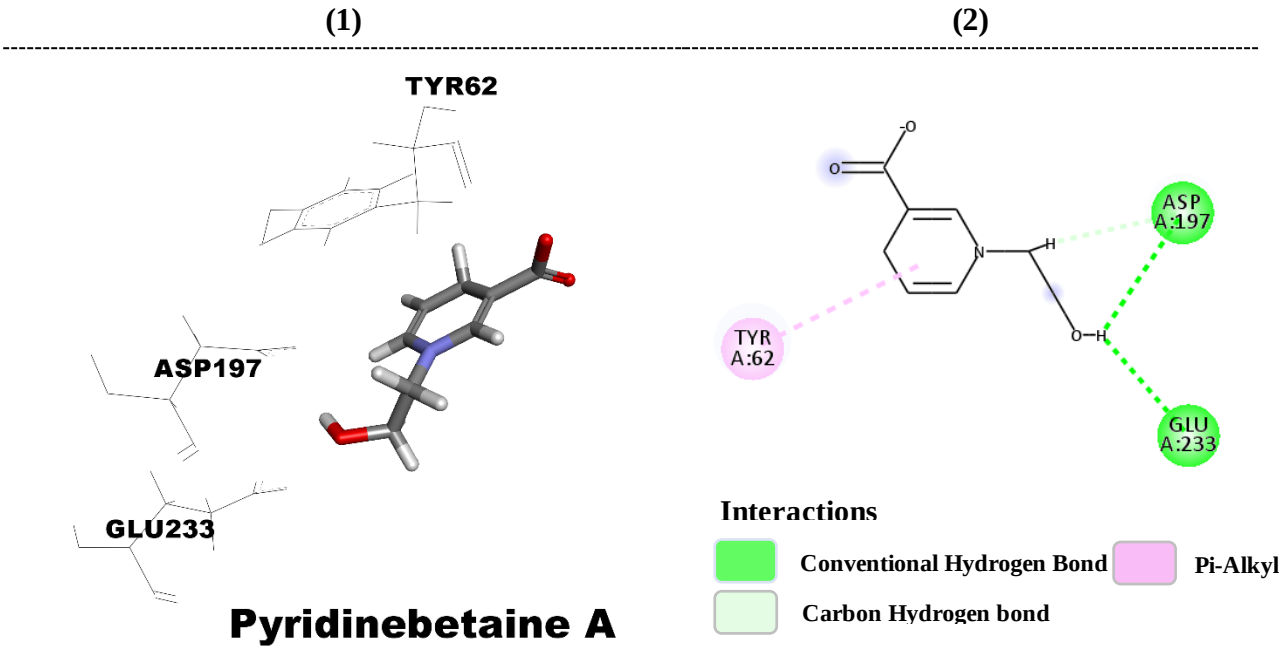


Figure 17. The 3D (**1**) and 2D (**2**) structures of the best pose after docking of ALK5 displayed in stick form and colored using default atom colors.

CONCLUSION

CONCLUSION

Type 2 diabetes mellitus, or T2DM, is a complex disease and poses a challenge in the search for its treatment. To date, there is no cure, although numerous drugs are used to delay carbohydrates catabolism by inhibiting human pancreatic α-amylase, and to reduce hyperglycemia in the blood after food intake.

Marine sponges produce a wide variety of secondary metabolites, some of which are alkaloids from certain sponge families. These compounds are often studied for their pharmacological and biological potential, such as cytotoxic, antibacterial, antifungal or antiviral properties.

To select marine sponge alkaloids as inhibitors with fewer side effects on human pancreatic α-amylase, a multi-step approach was used, including literature search, prediction of antidiabetic potential, pharmacokinetic profile assessment, and molecular docking.

Our *in silico* study shows that spongiacidin C (ALK3) from *Stylissa massa* was predicted as a possible HPA inhibitor with good Pa/Pi values and has significant HPA inhibition activity proven by its binding energy in addition to a good AMDT profile as its toxicity was minimal.

The other molecules (ALK1, ALK2 and ALK4) were considered moderate, as they were predicted to be HPA inhibitors with acceptable Pa/Pi values and high binding energies with an approved ADMT profile and low toxicity, while ALK5 was identified as a relatively weak inhibitor and ranked last in this work.

Marine sponge alkaloids are a new diabetes treatment source. Selected inhibitors show promise for T2DM therapy. *In vitro* and *in vivo* studies are needed for validation.

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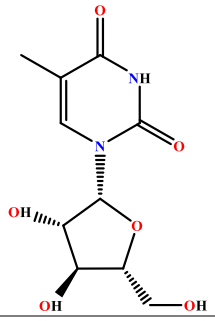
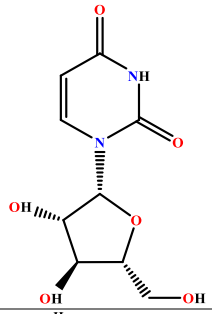
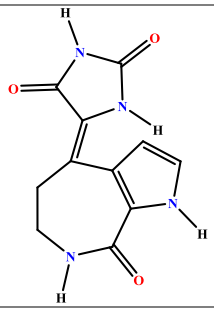
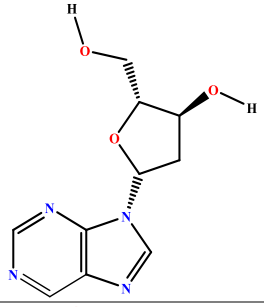
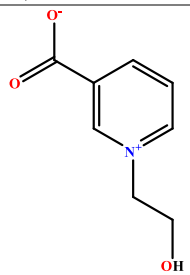
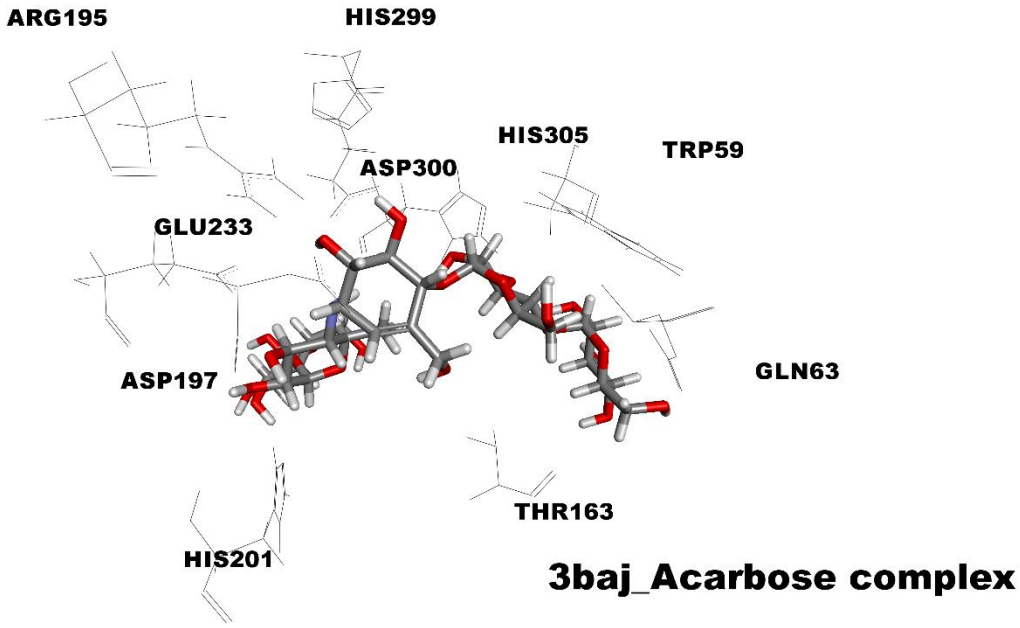
APPENDICES

Appendix 1. The 2D structure and IUPAC name of the five studied alkaloids.

Compounds	2D structure	IUPAC name
ALK1 **(Spongothymidine)**		1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione
ALK2 **(Spongouridine)**		1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidine-2,4-dione
ALK3 **(Spongiacidin C)**		(5Z)-5-(8-oxo-1,5,6,7-tetrahydropyrrolo[2,3-c]azepin-4-ylidene)imidazolidine-2,4-dione
ALK4 **(Deoxynebularine)**		(2R,3S,5R)-2-(hydroxymethyl)-5-purin-9-yloxolan-3-ol
ALK5 **(Pyridinebetaine A)**		1-(2-hydroxyethyl)pyridin-1-ium-3-carboxylate

Appendix 2. The 3D (**1**) and 2D (**2**) structures shows HPA-Acarbose complex after docking, displayed in stick form and colored using default atom colors.

(1)



(2)