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By:

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In silico study of the anti-amylase activity of certain metabolites derived from marine organisms

Publicly defended, on the 14/06/2026, before a jury composed of

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First of all, All praise and gratitude to Allah for granting me the health, power and courage to conduct this work.

I humbly dedicate this thesis

To my dear parents, my mother and my father, who have always provided me endless support, unconditional love, motivation and prayers throughout my studies, which have been the foundation of my success.

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To all my beloved family.

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Abstract

Diabetes mellitus is a prevalent chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion or reduced insulin activity in target tissues, which prevents the efficient utilization and storage of glucose. Although several antidiabetic medications are currently available, their adverse effects continue to limit therapeutic effectiveness. In this context, inhibition of human pancreatic α-amylase (HPA), a key enzyme involved in carbohydrate digestion, represents an important therapeutic strategy for the management of Type 2 Diabetes Mellitus (T2DM). The present study adopted a stepwise *in silico* strategy aimed at identifying safe and potent HPA inhibitors from marine-derived secondary metabolites. Initially, an extensive literature survey was conducted to collect compounds previously extracted, purified, and chemically characterized from marine organisms. The selected molecules were then subjected to safety filtering based on cardiotoxicity and carcinogenicity predictions in order to retain only safe compounds. Subsequently, the remaining safe candidates were evaluated through molecular docking against HPA using AutoDock Vina, while interaction analysis and visualization were performed with Discovery Studio Visualizer. The results revealed that several marine metabolites display strong binding affinity toward the catalytic site of HPA and exhibit favorable pharmacokinetic and safety profiles. Among them, Mol7, Mol22, and Mol23, derived from *Aspergillus* and *Streptomyces* species, demonstrated the highest inhibitory potential with binding energies of -10.0 kcal/mol and -9.5 kcal/mol, respectively. Importantly, all selected compounds showed stronger binding affinities than the reference inhibitor acarbose (-7.9 kcal/mol). These findings highlight the potential of marine-derived secondary metabolites as promising candidates for the development of novel HPA inhibitors for the treatment of T2DM. Nevertheless, further *in vitro* and *in vivo* studies are required to validate these computational predictions.

Keywords : Diabetes mellitus, HPA, T2DM, Molecular docking, *Aspergillus*, Acarbose.

Résumé

Le diabète sucré est un trouble métabolique chronique fréquent caractérisé par une hyperglycémie persistante due à une altération de la sécrétion ou de l'action de l'insuline dans les tissus cibles, empêchant l'utilisation et le stockage efficaces du glucose. Bien que plusieurs antidiabétiques soient disponibles, leurs effets indésirables limitent encore leur efficacité thérapeutique. Dans ce contexte, l'inhibition de l'α-amylase pancréatique humaine (HPA), enzyme clé de la digestion des glucides, constitue une stratégie thérapeutique importante pour la prise en charge du diabète de type 2 (T2DM). Cette étude adopte une approche *in silico* progressive visant à identifier des inhibiteurs non toxique et puissants de l'HPA parmi des métabolites secondaires d'origine marine. Dans un premier temps, une recherche bibliographique a permis de collecter des composés extraits, purifiés et caractérisés à partir d'organismes marins. Les molécules sélectionnées ont ensuite été filtrées selon des prédictions de cardiotoxicité et de cancérogénicité afin de ne retenir que les composés sûrs. Les candidats retenus ont ensuite été évalués par docking moléculaire contre l'HPA à l'aide d'AutoDock Vina, tandis que l'analyse et la visualisation des interactions ont été réalisées avec Discovery Studio Visualizer. Les résultats ont révélé que plusieurs métabolites marins présentent une forte affinité de liaison envers le site catalytique de l'HPA et affichent des profils pharmacocinétiques et de sécurité favorables. Parmi eux, Mol7, Mol22 et Mol23, dérivés des espèces *Aspergillus* et *Streptomyces*, ont démontré le potentiel inhibiteur le plus élevé avec des énergies de liaison de -10,0 kcal/mol et -9,5 kcal/mol, respectivement. Il est important de noter que tous les composés sélectionnés ont montré des affinités de liaison plus élevées que l'inhibiteur de référence, l'acarbose (-7,9 kcal/mol). Ces résultats mettent en évidence le potentiel des métabolites secondaires d'origine marine comme candidats prometteurs pour le développement de nouveaux inhibiteurs de l'HPA dans le traitement du diabète de type 2. Néanmoins, des études complémentaires *in vitro* et *in vivo* sont nécessaires afin de valider ces prédictions computationnelles.

Mots-clés : Diabète sucré, HPA, T2DM, Docking moléculaire, *Aspergillus*, Acarbose.

ملخص

يُعدّ داء السكري اضطرابًا أيضيًا مزمنًا شائعًا يتميّز بارتفاع مستمر في مستوى الغلوكوز في الدم نتيجة خلل في إفراز الإنسولين أو انخفاض فعاليته في الأنسجة المستهدفة، مما يعيق الاستفادة الفعّالة من الغلوكوز وتخزينه. وعلى الرغم من توفر العديد من الأدوية المضادة للسكري حاليًا، فإن آثارها الجانبية لا تزال تحدّ من فعاليتها العلاجية. وفي هذا السياق، يُعد تثبيط إنزيم ألفا-أميلاز البنكرياسي البشري (HPA) ، وهو إنزيم رئيسي يشارك في هضم الكربوهيدرات، استراتيجية علاجية مهمة في تدبير داء السكري من النوع الثاني. اعتمدت هذه الدراسة استراتيجية حاسوبية تدريجية تهدف إلى تحديد مثبطات آمنة وفعّالة لإنزيم HPA من بين المستقلبات الثانوية المشتقة من الكائنات البحرية. في البداية، أُجري مسح موسّع للأدبيات العلمية لجمع المركبات التي سبق استخلاصها وتنقيتها وتوصيفها كيميائيًا من الكائنات البحرية. ثم خضعت الجزيئات المختارة لعملية ترشيح أمني استنادًا إلى تنبؤات السمية القلبية وإمكانية التسرطن، بهدف الاحتفاظ بالمركبات الآمنة فقط. بعد ذلك، تم تقييم المرشحين الآمنين المتبقين باستخدام الإرساء الجزيئي ضد إنزيم HPAبواسطة برنامج **اوتودوك فينا**، في حين أُجري تحليل وتصور التفاعلات باستخدام برنامج **ديسكوفري**. أظهرت النتائج أن عدة مستقلبات بحرية تمتلك ألفة ارتباط قوية مع الموقع الفعال لإنزيم HPA، كما أظهرت خصائص دوائية حركية آمنة، ومن بينها، أظهرت المركبات Mol7 و Mol22 وMol23، المشتقة من *Aspergillus* و*Streptomyces*، أعلى قدرة تثبيطية بقيم طاقة ارتباط بلغت 10.0- و 9.5-كيلوكالوري/مول على التوالي. ومن الجدير بالذكر أن جميع المركبات المختارة أظهرت ألفة ارتباط أقوى من المثبط المرجعيAcarbose (7.9- كيلوكالوري/مول). تسلط هذه النتائج الضوء على الإمكانات الواعدة للمستقلبات الثانوية المشتقة من الكائنات البحرية كمصادر محتملة لتطوير مثبطات جديدة لإنزيم HPA لعلاج داء السكري من النوع الثاني. ومع ذلك، تبقى هناك حاجة إلى إجراء دراسات إضافية في المختبر وفي الكائن الحي للتحقق من صحة هذه التنبؤات الحاسوبية.

الكلمات المفتاحية :داء السكري، HPA، السكري من النوع الثاني، الإرساء الجزيئي، *Aspergillus*، Acarbose.

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A-B	C-D
ADMT: Absorption, distribution, metabolism, toxicity. **ADV:** AutoDock Vina. **ATP:** Adenosine triphosphate. **AD:** Anno Domini. **AGEs:** Advanced glycation end products. **AMPK:** Adenosine monophosphate-activated protein kinase. **ASCs:** Adult stem cells. **AMY1:** Alpha amylase 1. **AMY2:** Alpha amylase 2. **BC:** Before Christ. **BBB:** Blood-Brain Barrier.	**cAMP:** Cyclic adenosine monophosphate. **CSII:** continuous subcutaneous insulin infusion. **CGM:** continuous glucose monitoring. **CD3**: Cluster of Differentiation 3. **CD20:** Cluster of Differentiation 20. **Caco-2**: Human Colon Carcinoma Cell stage 2. **CYP:** Cytochrome. **CYA:** Czapek Yeast Autolysate Agar. **DSV:** Discovery Studio Visualizer. **DM:** Diabetes Mellitus. **DPP-4:** Dipeptidyl peptidase 4. **DAG:** diacylglycerol. **DNA:** deoxyribonucleic acid.
E-H	**I-L**
ESCs: Embryonic Stem Cells. **FDA:** Food and Drug Administration. **GAD:** Glutamic Acid Decarboxylase. **GLUT4:** Glucose Transporter 4. **GLUT2:** Glucose Transporter 2. **GLP-1:** Glucagon-like peptide 1. **GIP:** gastric inhibitory polypeptide. **GI:** Growth Inhibition **HERG:** Human Ether Related Gene. **HPA:** Human Pancreatic Alpha-amylase. **HLA:** Human Leukocyte antigen. **HIA:** Human Intestinal Absorption.	**IA-2:** Insulinoma-associated antigen 2. **iPSC:** induced Pluripotent Stem Cells. **ID:** Identifier. **IUPAC:** International Union of Pure and Applied Chemistry. **Kcal:** Kilocalorie. **KDa:** Kilo Daltons. **LEDP:** Plant Protection Expertise and Diagnostic Laboratory.
M-N	**P-R**
MAPAQ: Ministry of Agriculture, Fisheries and Food of Québec. **MDCK:** Mandin Darby canine kidney cell. **MD:** Molecular Docking. **MF:** Molecular Formula. **MW:** Molecular Weight. **MC:** Metabolite Class. **MOL:** Molfile. **MEA:** Malt Extract Agar. **MIC:** Minimum Inhibitory Concentration. **MRSA:** Methicillin-Resistant Staphylococcus Aureus. **NAG:** Nicotinamide adenine dinucleotide.	**PDB:** Protein Data Bank. **PDA:** Potato Dextrose Agar. **PPAR-γ:** Peroxisome Proliferator-Activated Receptor Gamma. **P-gp:** P-glycoprotein. **PPB:** Plasma Protein Binding. **PreADMET:** Prediction of Absorption, Distribution, Metabolism, Excretion, and Toxicity. **ROS:** Reactive Oxygen Species. **RNA:** Ribonucleic Acid. **RCSB:** Research Collaboratory for Structural Bioinformatics.

NCBI: National center for biotechnology information. **NMR:** Nuclear Magnetic Resonance.	
S-Z	
SDF: Structure Data Format. **SMILES**: Simplified Molecular Input Line Entry System. **SGLT2:** Sodium-Glucose Transport Protein 2. **SUR:** Sulfonylurea Receptor. **T1DM:** Type 1 Diabetes Mellitus. **T2DM:** Type 2 Diabetes Mellitus. **TSB:** Tryptic Soy Broth. **UCSF:** University of California, San Francisco. **V**: Version **YES:** Yeast Extract Agar. **ZnT8:** Zinc Transporter 8.	

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INTRODUCTION

Diabetes mellitus (DM) has been recognized since antiquity based on its symptoms rather than its pathogenesis and was described in several ancient medical texts, including Egyptian, Indian, Chinese, Greek, and Arab literature (Karamanou *et al.*, 2016). The Ebers Papyrus (≈1500 BC) reported polyuric syndromes associated with hyperglycemia, while Indian physicians referred to the disease as *madhumeha* "honey urine" because the urine attracted ants and flies, indicating glycosuria (Cavalli *et al.*, 2025). In the 2nd century AD, the Greek physician Aretaeus of Cappadocia introduced the term *diabetes*, meaning "siphon", to describe excessive urination (Cavalli *et al.*, 2025; Karamanou *et al.*, 2016). In the 17th century, Thomas Willis added the term *mellitus* "sweet", later explained by Matthew Dobson, who demonstrated the presence of sugar in diabetic urine (Cavalli *et al.*, 2025; Karamanou *et al.*, 2016). In 1869, Paul Langerhans described the pancreatic islets (Lakhtakia, 2013). Later, Frederick Banting and Charles Best discovered insulin in 1923, building on earlier experiments by Oskar Minkowski and Joseph von Mering, a discovery that revolutionized diabetes treatment (Mohajan, 2023).

Diabetes mellitus is diagnosed when blood glucose levels increase as a result of defects in insulin secretion or function, leading to the most common symptoms, which include polyuria, polydipsia, catabolic weight loss, hyperphagia and reduced visual acuity (Kashtoh & Baek, 2023). There are multiple lifestyle factors can support the risk of developing of this disease including smoking, obesity, genetic, ethnicity and drinking alcoholic beverages, an early management of these risk elements can prevent major complication (Alam *et al.*, 2021; Hossain *et al.*, 2024). There are two different types of diabetes mellitus, categorised based on their manifestation: type 1 diabetes mellitus (T1DM) is the result of pancreatic β cell destruction, while type 2 diabetes mellitus is characterised by insulin resistance (Kashtoh & Baek, 2023). Researchers have developed a new pharmacological method in the treatment of type 2 diabetes, based on inhibiting the digestion and the absorption of carbohydrates through human pancreatic alpha-amylase inhibitors, such as acarbose, miglitol, and voglibose, which are commonly used as hypoglycemic drugs (Febriyanti *et al.*, 2025).

The marine environment remains unexplored source of bioactive compounds produced by various organisms in response to extreme living conditions (Hang *et al.*, 2024). Marine organisms have demonstrated significant capacity for producing numerous natural compounds with powerful defensive mechanisms (Bettio *et al.*). Moreover, they exhibit several biological activities, including antibiotic, anticancer, anti-inflammatory and antiviral properties, which play a crucial role in drug discovery and disease treatment (Karthikeyan *et al.*, 2022).

This study aimed to investigate the antidiabetic potential of marine-derived compounds obtained from marine organisms including *Penicillium sp.*, *Talaromyces sp.*, *Aspergillus sp.*, and *Streptomyces sp.*, targeting human pancreatic α-amylase (E.C. 3.2.1.1) using computational approaches.

Initially, a literature search of *in vitro* studies was conducted to identify marine compounds that had been previously isolated, purified, and characterized. From 69 reported molecules, toxicity prediction was performed using the pre-ADMET 2.0 server (Gandla *et al.*, 2023) as a first screening step. Only compounds showing favorable predicted toxicity profiles, including acceptable hERG inhibition and non-carcinogenicity, were retained, resulting in 23 selected molecules.

Subsequently, molecular docking was carried out using AutoDock Vina via UCSF Chimera (Butt *et al.*, 2020) to predict the binding affinity of the selected compounds toward human pancreatic α-amylase (PDB ID: 3BAJ). As a second filter, only compounds showing better binding energy than the reference inhibitor acarbose were retained, leading to the selection of 12 promising candidates.

The study is organized into three main sections.

- The first section presents a literature review describing diabetes types, associated complications, current treatments, and the therapeutic relevance of human pancreatic α-amylase (HPA) as a molecular target, together with an overview of the studied marine organisms and their metabolites.

- The second section details the materials and methodology applied, followed by the presentation, analysis, and discussion of the obtained results.
- The final section summarizes the main findings and proposes perspectives for future research.

LITERATURE REVIEW

I. Diabetes mellitus

Diabetes mellitus (DM) is a complex metabolic disorder, characterized by hyperglycemia caused by the insufficient insulin production, secreted by beta cells of pancreas, or by a failure of insulin hormone to act in target organs (Hossain *et al.*, 2024; Szablewski, 2025), clinical symptoms intensity can varied with disease type and duration, and could affect all groups of ages, sexes, and regions worldwide (Antar *et al.*, 2023; Hossain *et al.*, 2024). In the early stage, patients may not exhibit any symptoms, which can contribute to multiple complications, if they are not treated properly (Antar *et al.*, 2023).

1. Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is characterised by increased blood glucose levels due to an autoimmune disorder that leads to the destruction of pancreatic β-cells in the islets of Langerhans (Figure 1). These cells are responsible for producing insulin, the main hypoglycaemic hormone that maintains blood glucose levels. Therefore, patients with T1DM are dependent on exogenous insulin (Benkahla *et al.*, 2021).

Genetic factors are a crucial cause of T1DM pathogenicity, although environmental factors could also be responsible (Xie *et al.*, 2014). Over 50 genes have been elucidated to be associated with T1DM within the Human Leucocyte Antigen (HLA) class II gene. HLA class II molecules are responsible for presenting extracellular antigens to T cells (lymphocytes) and are found on antigen-presenting cells (Z. Wang *et al.*, 2017). The precise mechanism by which this gene leads to β-cell destruction remains unclear, but it is hypothesised that their peptide-binding properties may influence antigen presentation, thereby contributing to autoimmunity through the activation of the immune system and the production of autoantibodies, particularly against epitopes derived from proinsulin, insulinoma-associated antigen 2 (IA-2), glutamic acid decarboxylase (GAD) and zinc transporter 8 (ZnT8), which are several islet cell self-antigens (Pugliese, 2017; Xie *et al.*, 2014).

Environmental factors, particularly viral infections, are thought to be major factors associated with T1DM. One theory is that molecular mimicry occurs, whereby the virus presents similar epitopes to those found on the beta cells. This provides activation of T cells and induces a cross-reactive autoimmune response (Xie *et al.*, 2014).

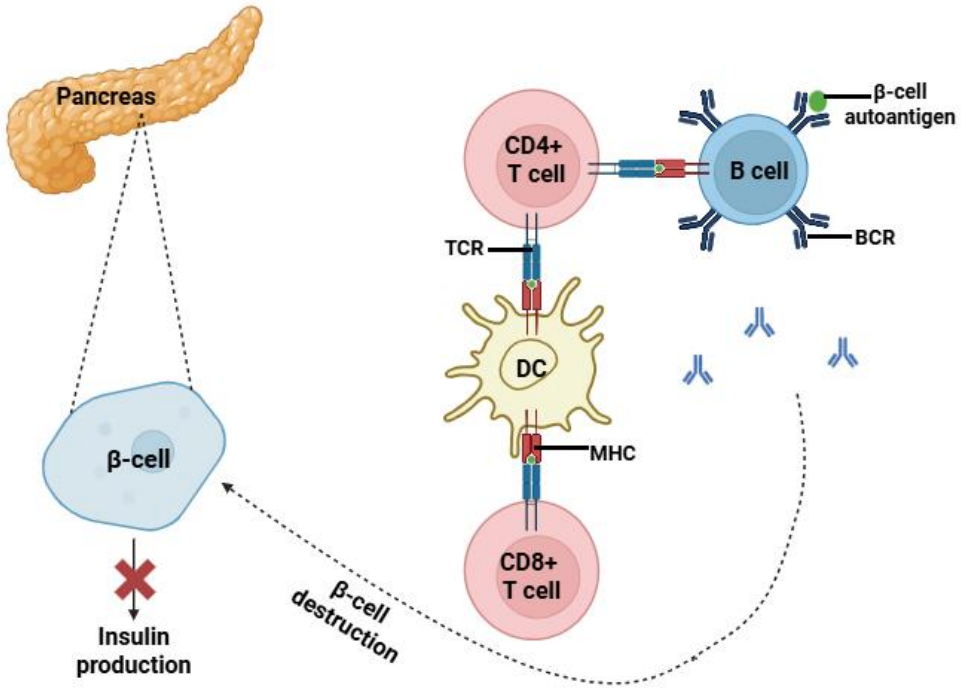


Figure 1: The immune system, particularly T and B lymphocytes, mistakenly attacks and destroys pancreatic β-cells (Harrat, 2024; Katsarou *et al.*, 2017).

2. Type 2 diabetes mellitus

Type 2 diabetes (T2DM) is a common chronic forms of diabetes with a multifactorial etiology (Figure 2), both of genetic and environmental factors (Młynarska *et al.*, 2025), the prevalence have demonstrated that this type of diabetes which accounts for 90-95% of all patients worldwide, can strongly affect older people but also, to a lesser extent, younger adults (Chandrasekaran & Weiskirchen, 2024; Młynarska *et al.*, 2025).

T2DM is commonly progresses slowly and manifest with these symptoms, such as polyuria, polyphagia, excessive thirst, fatigue, and weight loss (Ortiz-Martinez *et al.*, 2022; Singh *et al.*, 2025), this disorder occurs when pancreatic cells do not secrete sufficient insulin, or when

targeted tissue resist to insulin's action, resulting in a high level of glucose in blood, and leading to multiple serious complications (Młynarska *et al.*, 2025). The insulin resistance may develop from genetic alternations in glucose transporters, insulin receptors, or components of the insulin signaling pathway (Lu *et al.*, 2024; Młynarska *et al.*, 2025).

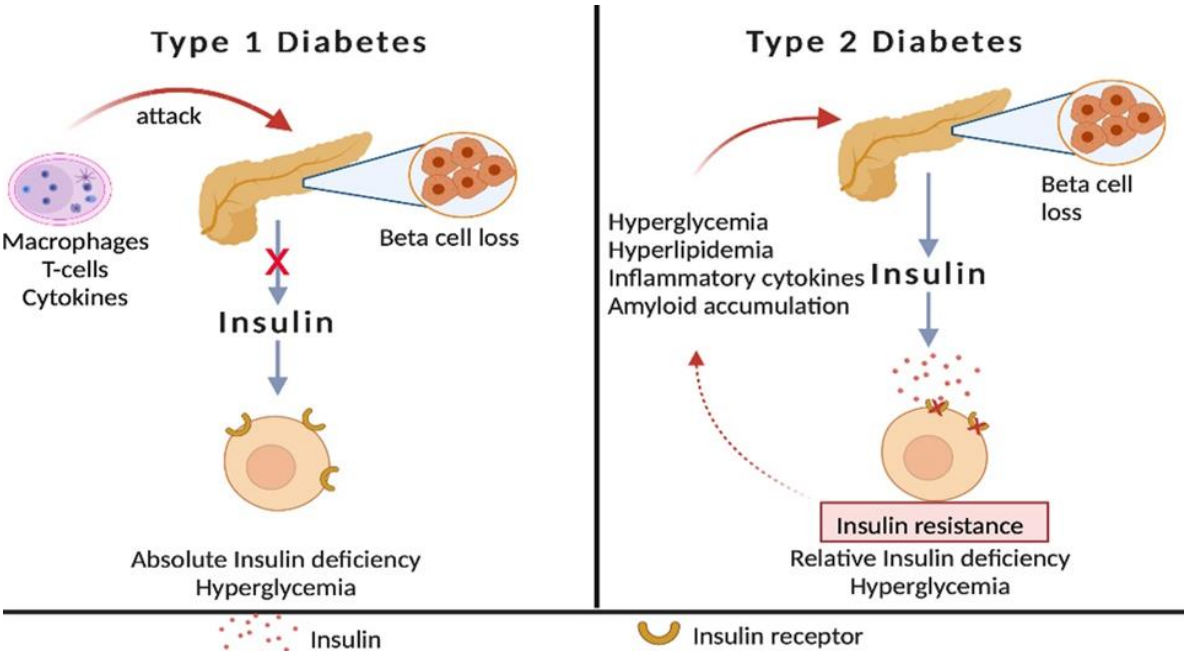


Figure 2: The distinction between Type 1 diabetes mellitus and Type 2 diabetes mellitus (Harrat, 2024; Khin *et al.*, 2023).

3. Diabetes-related complications

Patients with type 1 diabetes suffer from a number of complications due to high blood glucose levels over a long period. These complications are categorised as macrovascular, characterised by damage to large blood vessels and leading to cardiovascular diseases, or microvascular, such as retinopathy (eye problems), neuropathy (nerve damage) and nephropathy (kidney failure) (Verma *et al.*, 2021). Several biological processes are implicated in the onset of micro and macrovascular complications:

- The formation of advanced glycation end products (AGEs) through non-enzymatic glycosylation of proteins, which alters the function of proteins and leads to cardiovascular and renal complications.

- The activation of several signalling pathways in endothelial cells, smooth muscle cells, macrophages and T cells leads to different responses and the production of ROS as a consequence of AGE binding to cell surface receptors.
- The activation of protein kinase C as a result of diacylglycerol (DAG) accumulation leads to cardiovascular complications via different processes.
- The activation of the polyol pathway as a result of hyperglycaemia leads to peripheral nerve damage (Saberzadeh-Ardestani *et al.*, 2018).

4. Diabetes treatment

There are many strategies for managing T1DM and T2DM. However, while the current drugs help to balance blood glucose levels, a cure has yet to be found. [45-47].

4.1 Type 1 diabetes mellitus

- **Injected insulin**

The administration of insulin to patients with T1DM can be managed using several delivery systems (Figure 3). Patients usually require three to four injections of insulin per day using syringes and vials, and sometimes mix multiple insulin preparations to achieve the desired biological effects. Pens are easier to handle than syringes and provide precise dose adjustment, improving glycemic control. They combine the insulin reservoir and injection mechanism into a single device (Al-Tabakha & Arida, 2008; Shah *et al.*, 2016). Offering greater flexibility and comfort to patients in their daily lives, insulin pumps, also known as continuous subcutaneous insulin infusion (CSII), provide an alternative treatment option for T1DM. They couple an insulin reservoir to a subcutaneous catheter via plastic tubing, or in the case of patch pumps, wirelessly (Al-Tabakha & Arida, 2008; Berget *et al.*, 2019). Whatever treatment method is used, patients need to regularly monitor their blood glucose levels using a blood glucose meter (Zhang *et al.*, 2019).

Figure 3 : Various devices employed for insulin injection.

- **Inhaled insulin**

The most promising alternative to injected insulin is the inhaled one, which present potentially effective treatment for patients with T1DM. It can be administrated in a dry powder formulation or in solution using different patented inhaler systems (Al-Tabakha & Arida, 2008).

4.2 Type 2 diabetes mellitus

Several medications are currently available for the treatment of type 2 diabetes mellitus (T2DM) by oral administration, including biguanides, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter-2 (SGLT2) inhibitors and Alpha-glucosidase and α-amylase inhibitors (Mathu *et al.*, 2021).

- **Biguanides**

Biguanides are a class of oral anti-hyperglycaemic drugs, with metformin being the most commonly prescribed for treating type 2 diabetes mellitus (T2DM). It increases the liver's sensitivity to insulin and reduces glucose levels in the bloodstream by acting on complex I of the mitochondrial respiratory chain, which leads to the inhibition of gluconeogenesis and the activation of AMPK, as well as an increase in cAMP levels and a decrease in ATP levels (Jones *et al.*, 2022).

Sulfonylureas

Sulfonylureas stimulate insulin secretion from pancreatic cells (Figure 4), leading to a decrease in blood glucose levels. Among this class, Glipizide is one of the most widely used drug, it binds to the sulfonylurea receptor (SUR) present in the membrane of β-cells. This interaction modulates ATP-sensitive potassium (K_(ATP)) channels, inhibiting potassium ion efflux and leading to membrane depolarisation and an increase in Ca^{2+} levels, which causes insulin release (Tomlinson *et al.*, 2022).

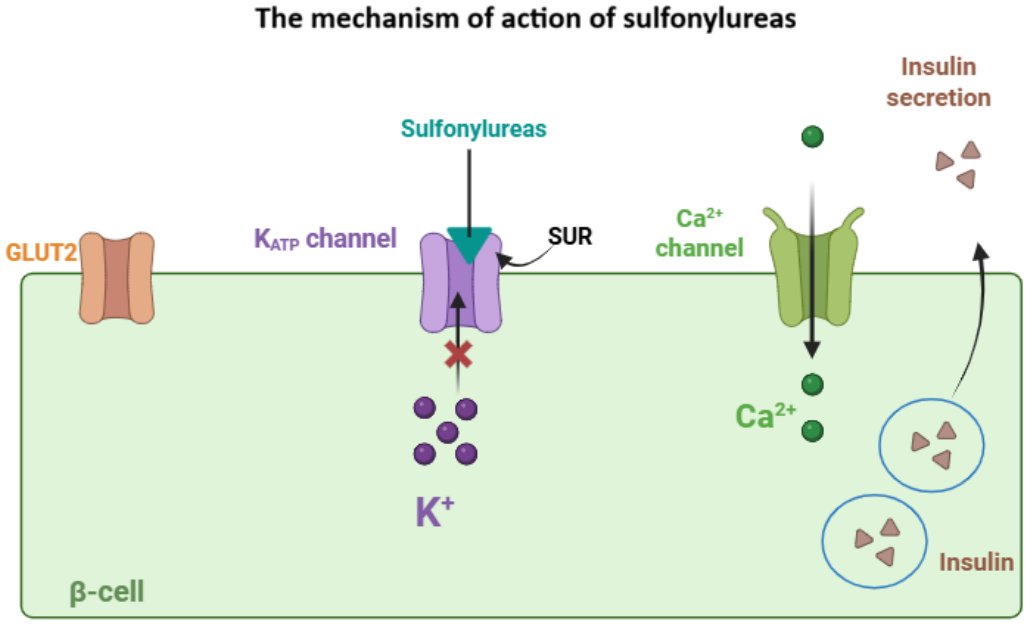


Figure 4: The mechanism of action of sulfonylureas in pancreatic cells.

Thiazolidinediones

Thiazolidinediones are a well-documented class of oral anti-diabetic agents (Gurvinder *et al.*, 2024), that increase β-cell sensitivity to glucose levels and reduce insulin resistance by activating the nuclear transcription factor PPARγ (peroxisome proliferator-activated receptor gamma), which affects gene expression in peripheral tissues such as the liver and skeletal muscle (Susilawati *et al.*, 2023). This activation increases the expression of GLUT4 receptors and glucose metabolism (Genovese, 2023).

Sodium-glucose co-transporters 2 inhibitors

Sodium-glucose co-transporters 2 (Figure 5) are a group of transporters, found in the proximal tubules of kidney cells, responsible for the reabsorption of filtered glucose and sodium into the blood circulation, this process is coupled with the Na-K-ATPase pump and GLUt2 mediator (Xu *et al.*, 2022). In the past few years, the FDA has selected three main SGLT2 inhibitors known as gliflozins, such as empagliflozin, canagliflozin and dapagliflozin, they have been shown to decrease plasma sugar concentration by inhibiting renal reabsorption, thereby increasing glucose levels in urine. This mechanism helps to control glucose homeostasis levels and prevent hyperglycemia (Fonseca-Correa & Correa-Rotter, 2021).

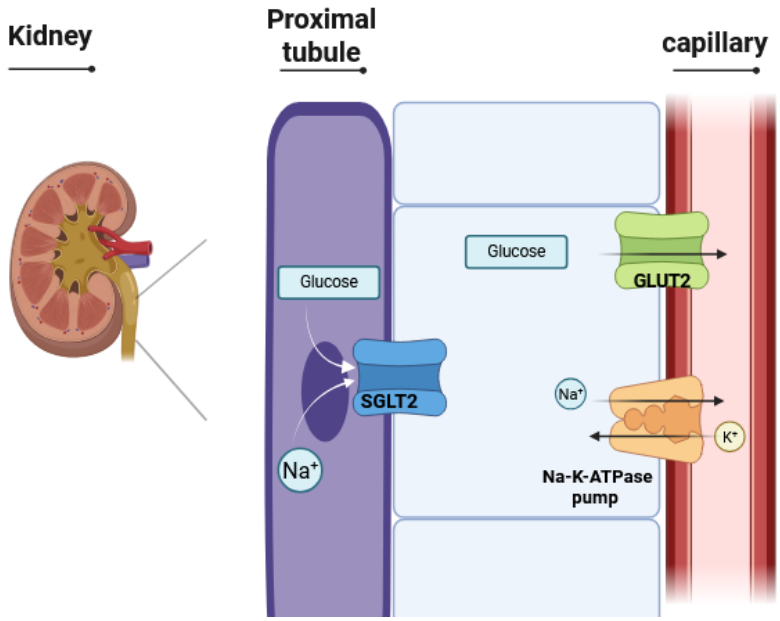


Figure 5: Mechanistic action of sodium-glucose cotransporter 2 (SGLT2) in the reabsorption of glucose under normal physiological conditions (Ahwin & Martinez, 2024; Chao & Henry, 2010; Harrat, 2024).

DPP-4 inhibitors

Dipeptidyl Peptidase 4 (DDP-4) is an enzyme derived from a family of proteases, that is predominantly exists in endothelial and epithelial cells, it plays a central role in the regulation of glucose metabolism by involving the breakdown of gastrointestinal incretin hormones, such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), which are

released upon food intake from the K-cells and L-cells, respectively (Epelde, 2024; Saini *et al.*, 2023). Gliptins are a set of oral agents that lower the activity of the DPP-4 enzyme (Figure 6), so they help rising the quantity of active incretins, resulting in increased insulin and inhibited glucagon secretion, ultimately they maintain glucose hemostasis (Shao *et al.*, 2024). The most popular medications used are sitagliptin and linagliptin, due to their favorable safety and tolerability profile, making them a good candidates for the treatment of blood sugar levels (Gallwitz, 2019).

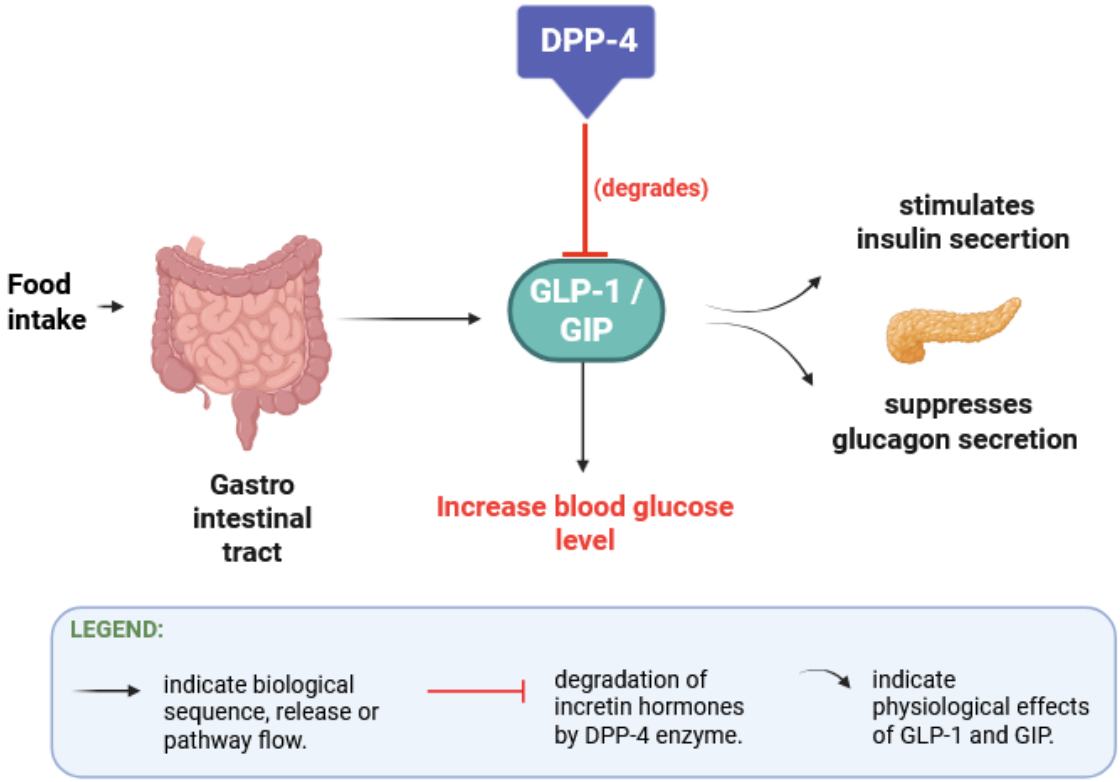


Figure 6: Mechanism of DPP-4 degradation of gastrointestinal incretin hormones (Shaikh *et al.*, 2021).

Alpha-glucosidase and α-amylase inhibitors

Alpha-glucosidase and α-amylase are digestive enzymes that degrade carbohydrates, which are considered as the principal source of dietary calories. During food digestion, starch is extensively hydrolyzed through α-amylase activity into smaller oligosaccharides and disaccharides, which are further broken down into glucose by the action of α-glucosidase

(Ćorković *et al.*, 2022). The FDA has approved Acarbose, Voglibose, and Miglitol as potent compounds, that showed an inhibitors effect against these enzymes, which are helpful in minimizing the degradation of polysaccharides and the reduction of hyperglycemia (Khan *et al.*, 2024).

4.3 The artificial pancreas

The traditional daily insulin injection was frequently challenging for patients with diabetes. The artificial pancreas was the most technological alternative system, that used to control blood glucose levels (Figure 7), this advanced device knows as closed-loop systems, includes a continuous glucose monitoring (CGM) and continuous subcutaneous insulin infusion (CSII) linked by a control algorithm. The CGM works by inserting a sensor in the subcutaneous tissue and detecting glucose concentrations every 5-10 minutes, afterwards, the controller wirelessly transmits CGM signals to the insulin pump (CSII) which delivers the required amount of insulin into the interstitial fluid (Moon *et al.*, 2021; Nwokolo & Hovorka, 2023).

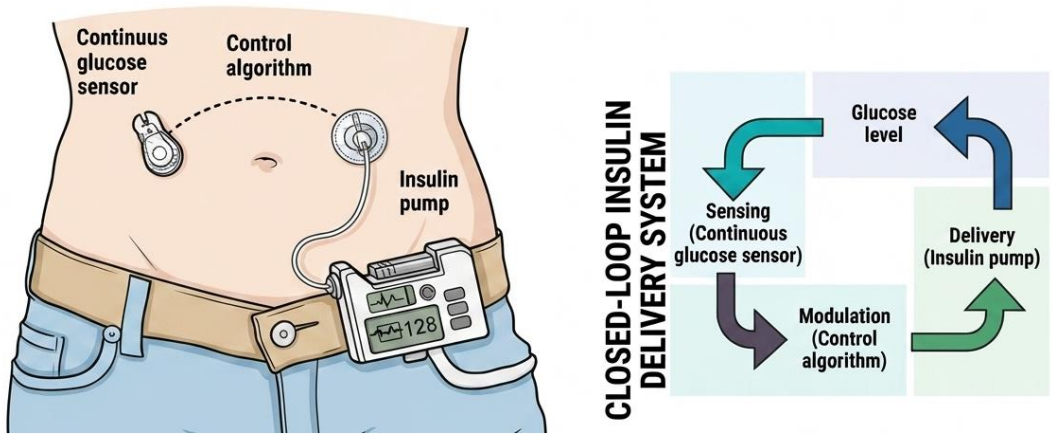


Figure 7: Closed-loop of artificial pancreas systems (Nwokolo & Hovorka, 2023).

4.4 New approaches

- **Stem cell therapy**

Stem cells are non-specialized cells with great potential for differentiation, regeneration and self-renewal into several somatic cells and tissue types, therefore they are used as a new and promising therapeutic method for treating diabetes mellitus disease (Figure 8) and other disorders (Mousaei Ghasroldasht *et al.*, 2022).

The stem cells are categorized into three principal forms that are widely used in disease treatment and drug screening applications like:

- Human embryonic stem cells (ESCs) (Totipotent stem cells).
- Induced pluripotent stem cells (iPSCs).
- Adult stem cells (ASCs) (multipotent stem cells) (Rahimi Darehbagh *et al.*, 2024; Wang *et al.*, 2024).

These cells offer a potential therapeutic solution by replacing damaged pancreatic beta cells in patients with diabetes, via a process known as cell transplantation (Pagliuca *et al.*, 2014).

In vitro, scientists have managed to regenerate unlimited number of beta cells using iPSCs, which are derived from adult stem cells that have been genetically altered and reprogrammed through various genetic factors. Consequently, they exhibit properties comparable to those of embryonic stem cells (ESCs) and can differentiate into multiple specialized cell types (Silva *et al.*, 2022).

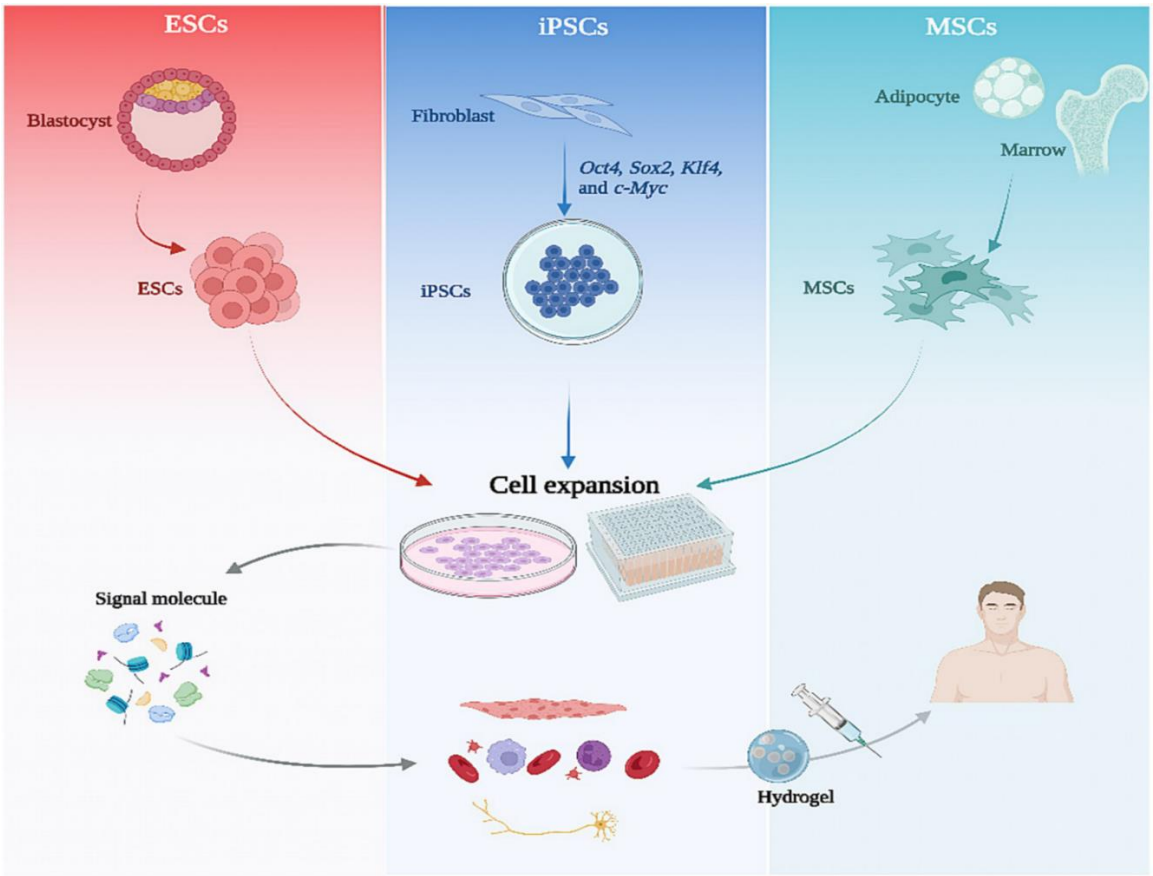


Figure 8: Overview of different stem cells therapy pathway (Wang *et al.*, 2024).

- **Immunotherapy**

The destruction of the pancreatic beta cells in type 1 diabetes mellitus is an autoimmune problem driven by T cells, leading to a deficiency insulin production. Currently immunotherapy has become a central approach to preventing the progression of DM, by targeting certain components of the immune system, particularly T cells, B cells and cytokines. Teplizumab, an anti-CD3 monoclonal antibody and Rituximab, anti CD20 antibody, are widely used as an immunomodulatory drug in individuals with diabetes (Salame *et al.*, 2025; Sann *et al.*, 2024).

- **Gene therapy**

The treatment of T1DM using this approach is still being explored in animal models. This technique involves correcting the defective gene by modifying, inactivating or introducing a new gene using viral vectors (Chellappan *et al.*, 2018).

II. Human pancreatic amylase

The pancreas plays an important role in bodily functions through its endocrine and exocrine functions, making it a key internal organ. The endocrine function involves the secretion of multiple hormones, while the exocrine function involves the production of several enzymes responsible for food digestion, such as lipase and phospholipase for fat digestion, and amylase for carbohydrate digestion. This alkaline pancreatic juice passes to the duodenum to fulfil its role (Karpińska & Czauderna, 2022).

The α-amylase (E.C. 3.2.1.1) is an enzyme that plays an important role in the catabolism of carbohydrates into oligosaccharides and disaccharides, by acting on the α(1,4) glycosidic bonds of polysaccharides, it is primarily found in saliva and the small intestine (Devi *et al.*, 2025), the salivary amylase is encoded by the AMY1 gene, whereas pancreatic amylase (HPA) (E.C. 3.2.1.1) is encoded by the AMY2 gene, both genes are presented on the short arm of chromosome 1, these two enzymes are the main isozymes of α-amylase, and differ by approximately 3% in their amino acids residue (Butterworth *et al.*, 2011; Date *et al.*, 2020; Kalinovskii *et al.*, 2023).

1. 3D Structure information

HPA is a large protein comprising 496 amino acids arranged in a single polypeptide chain, it has a molecular weight of 56 kDa and requires two essential ions for its activity, namely calcium and chloride ions (Brayer *et al.*, 2000; Rydberg *et al.*, 1999), the crystal structure of HPA demonstrates three different domains: A, B and C (Figure 9).

- **Domain A:** The largest catalytic domain is made up of two segments, the first one begins at residue 1 to 99, and the second extends from 169 to 404, It contains eight-stranded β-barrel and α-helical segments. This biggest domain contains the catalytic triad residues ASP 197, GLU 233, and ASP 300. Additionally, chloride ion Cl^- interacts with ARG 195, ASN 298, and ARG 337 residues found in this domain.
- **Domain B:** is the smallest domain with only 63 amino acids (residues 100-163), it binds with calcium ion through ASN100, ARG 158, ASP 167, and HIS 201, all of which play a central role in stabilizing the active site.
- **Domain C:** it starts from residue number 405 to 496 and consists of an anti-parallel beta-structure, It is involved in the binding of starch to the amylase enzyme (Brayer *et al.*, 1995; Qin *et al.*, 2011; Rydberg *et al.*, 1999).

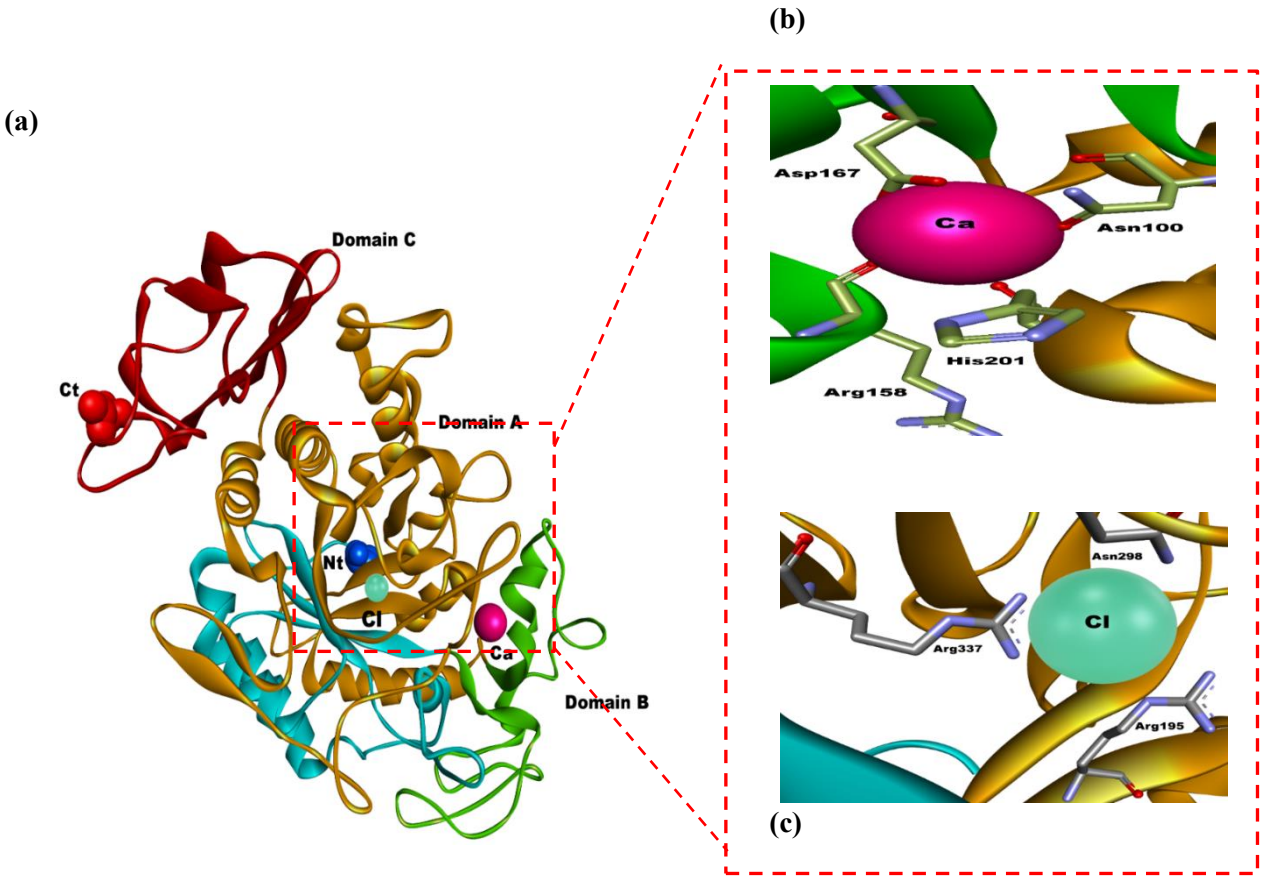


Figure 9: (a) The optimized 3D structure of human pancreatic α-amylase, showing its three domains. Domain A is depicted in pale cyan and orange, Domain B in green, and Domain C in red, while the N-terminal (Nt) and C-terminal (Ct) regions are highlighted in blue and red, respectively. (b) Illustration of the Ca^{2+} binding site. (c) Representation of the Cl^- binding site, PDB ID:3BAJ.

2. Mechanism of action

The activity of amylase is demonstrated by the double-displacement mechanism (Figure 10), in which two aspartates (ASP197 and ASP300) and a glutamate (GLU233) are involved in the catalytic process (Whitcomb & Lowe, 2007). Throughout the reaction, GLU233 (an acid catalyst residue) and ASP197 (a nucleophilic amino acid) participate in the cleavage of the glycosidic bond, whereby Glu233 donates a proton and ASP197 attacks the bond, resulting in the formation of a covalent intermediate. Subsequently, water restores the proton to glutamate and adds a hydroxyl group to the remaining substrate. ASP300 indirectly assists by maintaining the position of the substrate (Samanta, 2022).

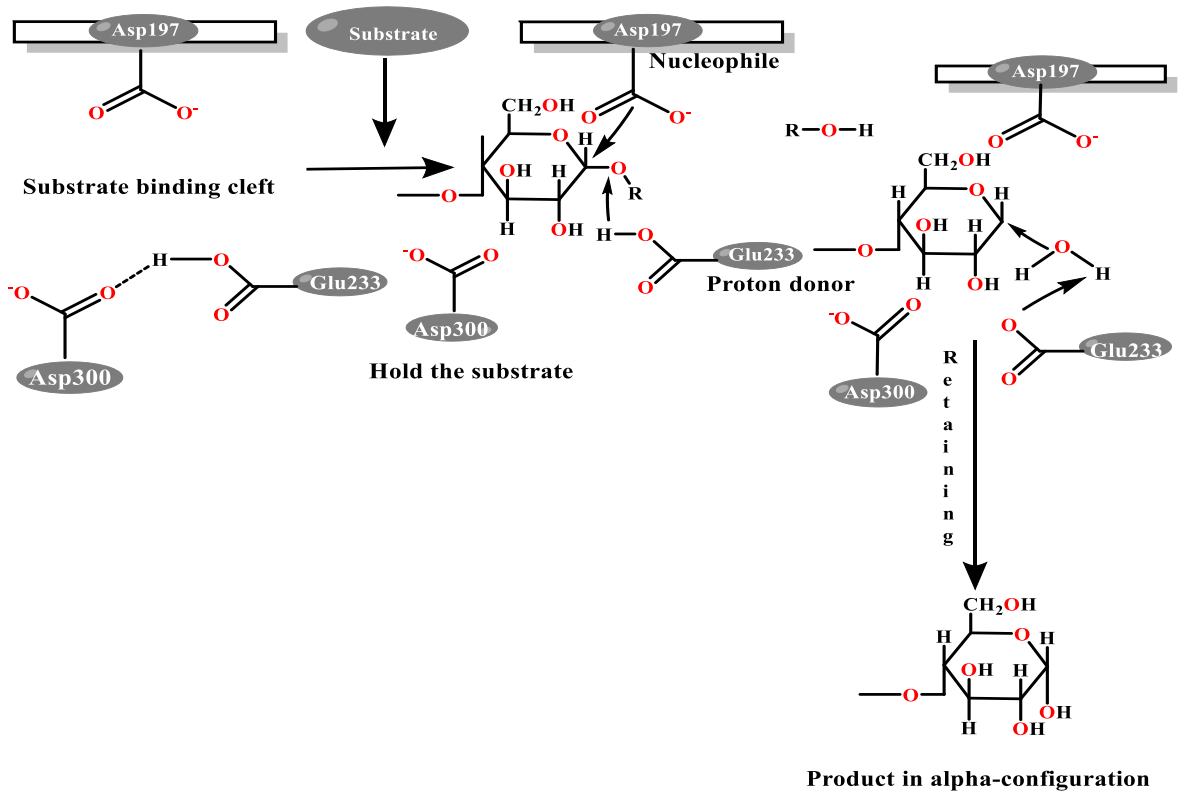


Figure 10: Catalytic mechanism of HPA during starch digestion (Harrat, 2024; Samanta, 2022).

III. Studied marine organisms

Secondary metabolites have gained a great interest and have become exclusive natural sources in various therapeutic and pharmaceutical applications, due to their significant biological and structural proprieties (Hussain *et al.*, 2012). A large number of these chemical compounds were initially identified in plant species, which lead to multidimensional purposes, especially in defending against predators, protecting from toxic effects, and transmitting signals in cells, and they are considered as the most contributors of sensory properties of plants (Ahmed *et al.*, 2017).

Over the past decades, the isolation of naturals products from marine environment has turned out to an interest field and a mature source due to the presence of enormous bioactive compound with approximatively of 28,500 molecules from different marine organisms such as macroalgae, octocorals, sponges, bacteria, and fungi (Barzkar *et al.*, 2023; Kelecom, 2002). Nowadays marine organisms are receiving remarkable attention as producers of myriad

secondary metabolites with a potent anticancer, antioxidant, antibacterial and anti-inflammatory activities, which are anticipated to be used in drug discovery process as well as in biotechnology sector (Petersen *et al.*).

Within this research we represent three fungal species: *Penicillium sp, Talaromyces sp, Aspergillus sp* and one bacterial species *Streptomyces sp* based on their capacity to produce a broad range of therapeutic metabolites. Herein, we present the taxonomic classification of these species obtained from the NCBI website, along with their geographical distribution and morphology.

1 Marine fungi

1.1 *Penicillium sp*

1.1.1 Taxonomy

Kingdom	***Fungi***
Phylum	***Ascomycota***
Class	***Eurotiomycetes***
Order	***Eurotiales***
Family	***Aspergillaceae***
Genus	***Penicillium***

1.1.2 Geographical distribution and morphology

Penicillium species (Figure 11) are highly distributed fungal organisms, more than 350 species on different environmental habitats. Marine *Penicillium* fungal genera can produce abundant biologically active secondary metabolites classes, such as alkaloids, polyketides, and terpenoids, which can play a crucial role in the discovery and development of new therapeutic and pharmaceutical drugs (Chen *et al.*, 2022).

The species belonging to this genus are widely distributed in diverse marine regions including Korea (Lee *et al.*, 2025), Portugal seas (Gonçalves *et al.*, 2019), Nha Trang Bay and Van Phong Bay in Vietnam (Nguyen & Pham, 2022), and Zostera marina in Japan (Afiyatullov *et al.*, 2017). They are defined macroscopic characteristics such as velutinous, floccose, or powdery

textures in different colors found in the colony, and by microscopic features, particularly brush-like structure conidiophores.

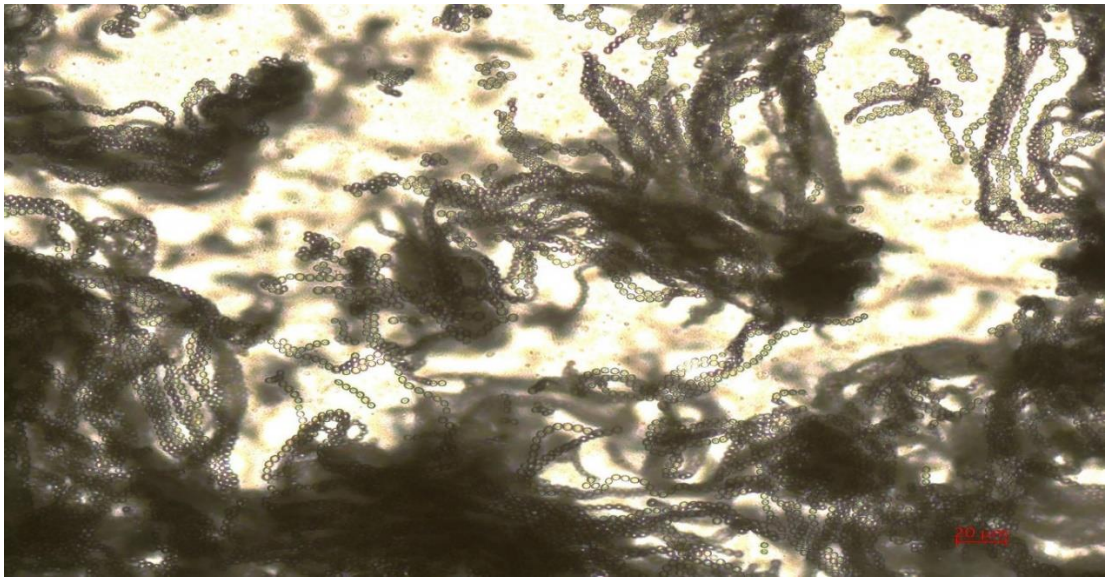


Figure 11: Microscopy observation of *Penicillium* conidial chains.

Image source: Plant Protection Expertise and Diagnostic Laboratory (LEDP), Ministry of Agriculture, Fisheries and Food of Québec (MAPAQ), Canada.

1.1.3 Derived metabolites

Numerous bioactive compounds have been obtained from marine natural fungi, especially the *penicillium* genus which has received a great attention from the scientific community in the discovery for new drugs. one of the notable metabolites isolated from this genus, is 2-[(5-methyl-1,4-dioxan-2-yl) methoxy] ethanol, which present a significant inhibitory effect against the Gram-positive bacterium *Enterococcus faecalis* and the fungus *Candida albicans* with a MIC value of 32 µg/mL and 64 µg/mL, respectively.

4-hydroxybenzandehyde is another natural product, that exhibit an inhibitory effect against *Escherichia coli*, with MIC values of 8 µg/mL (Le *et al.*, 2019). Penialidin A, Oxypenicinoline A, and austalide P, have showed substantial α-glucosidase inhibitory activity, with inhibition rates of 36.9% at 400 µM, 317.8 µM and 910 µM respectively (Zimin Wang, Meirong Zhao, Yunxia Yu, *et al.*, 2025). In addition, penicillol B is another polyketide that plays a role as anti-inflammatory substance in reducing the production of nitric oxide with an IC_{50} value of 12 µM (Chen *et al.*, 2022).

1.2 *Aspergillus sp*

1.2.1 Taxonomy

Kingdom	***Fungi***
Phylum	***Ascomycota***
Class	***Eurotiomycetes***
Subclass	***Eurotiomycetidae;***
Order	***Eurotiales***
Family	***Aspergillaceae***
Genus	***Aspergillus***

1.2.2 Geographical distribution and morphology

Species belonging to the *Aspergillus* section are easily identifiable by their distinctive colony and microscopic features (Figure 12). Colonies typically exhibit yellowish hyphae with profuse aerial growth, while the conidial heads are greenish-yellow and often have a radiating structure. Some isolates may also exhibit reddish pigmentation of the hyphae (Lee *et al.*, 2016).

Marin *Aspergillus* is generally associated with various marine organisms, such as algae and sponges, and has been isolated from diverse marine ecosystems. Samples of this fungus were obtained from the western and southern coasts of Korea (Lee *et al.*, 2023; Lee *et al.*, 2016), as well as the Pichavaram mangrove and the Valliyar estuary in the Indian state of Tamil Nadu (Thiyagarajan *et al.*, 2016).

Figure 12: Photo represents Aspergillus sclerotiorum (Zimin Wang, Meirong Zhao, Yunxia Yu, *et al.*, 2025).

1.2.3 Derived metabolites

Marine *aspergillus* is an important source of various organic compounds, such as terpenoids, alkaloids and polyketones, which have distinct structures and exhibit diverse biological activities (Sun *et al.*, 2022). Aspergillosteroid A, a steroid isolated from strain LS116, showed significant antibacterial activity against the aquatic pathogen Vibrio harveyi, with an MIC value of 16 μg/mL, making it a new potential lead compound for managing aquatic diseases (Xu *et al.*, 2020).

In addition, asperterphenylcin B, a polyketide obtained from this genus, demonstrated significant inhibitory activity against α-glucosidase, with an IC_{50} value of 1.3 ± 0.2 μM (Zimin Wang, Meirong Zhao, Chenglin Li*, et al.*, 2025).

Versicolactone G also exhibited potential inhibitory activity against α-glucosidase, with an IC_{50} value of 104.8 ± 9.5 μM, which was lower than the positive control, acarbose (IC_{50} = 154.7 ± 8.1 μM) (Liu *et al.*, 2018).

1.3 *Talaromyces sp*

1.3.1 Taxonomy

Kingdom	***Fungi***
Phylum	***Ascomycota***
Class	***Eurotiomycetes***
Order	***Eurotiales***
Family	***Trichocomaceae***
Genus	***Talaromyces***

1.3.2 Geographical distribution and morphology

Talaromyces is a fungal (Figure 13) genus belonging to the phylum *Ascomycota*, they are widely inhabited in various terrestrial habitats and aquatic ecosystem (Ren *et al.*, 2024). Is an important natural resource producing plenty of biologically active compounds , including enzymes and diverse secondary metabolites with promising potential for agricultural, medical, and pharmaceutical fields (Nguyen & Lee, 2023). Morphologically, species within this genus

are typically characterized by the formation of ascomata with creamish, yellow, white, pink, or reddish color, and also produce yellow ascospores, the majority of talaromyces species have biverticillate conidiophores, while some of them have both biverticillate and monoverticillate conidiophores structures (Nguyen & Lee, 2023).

Strain BTBU20213036 was isolated from the intertidal regions of the Yellow Sea in Qingdao, China (Song *et al.*, 2022) and from Hwangnyong river ,Gwangju,in Korea (Nguyen & Lee, 2023). Similary, another fungal strain of *Talaromyces mangshanicus* BTBU20211089, was obtained from a marine sediment sample in the South China Sea (Zhang *et al.*, 2022), this strain shows a slow growth with an irregular colony , absent or limited sporulation and no germination on CYA agar, biverticillate conidiophores, ampulliform phialides, and echinulate subglobose-ellipsoidal conidia (X.-C. Wang *et al.*, 2017).

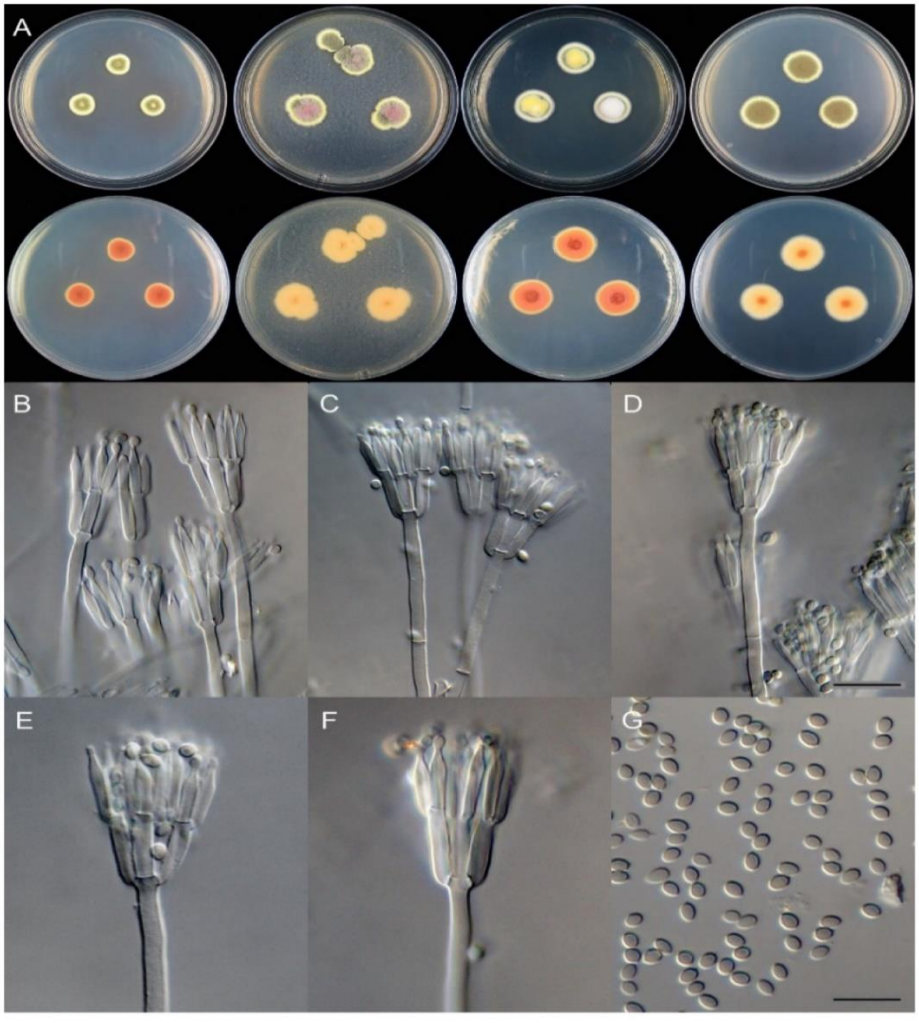


Figure 13: *Talaromyces chongqingensis* (CS26-67).

(A) Colonies: top row left to right, obverse CYA (Czapek yeast autolysate agar), MEA (malt extract agar), YES (yeast extract agar), and PDA (potato dextrose agar); bottom row left to right, reverse CYA, MEA, YES, and PDA; (B-F) Conidiophores; (G) Conidia. Bars: (D) 15 µm, applies also to (B,C); (G) 10 µm, applies also to (E,F) (Zhang *et al.*, 2021).

1.3.3 Derived metabolites

Talaromyces represent a paramount source of various secondary metabolites with an extraordinary biological activity, among them two anti-inflammatory alkaloids azaspirofurans A and fumiquinones B, which have been isolated from this marine fungus, exhibit a remarkable nitric oxide inhibitory effect, with IC50 values of 9.65 and 15.54 µM, respectively (Cong *et al.*, 2022). Furthermore, *Talaromyces* produce another natural product called peniciisocoumarin D, that shows strong antioxidant activity with IC_{50} value below 0.16 mM, moreover it has a potent inhibitory properties against α-glucosidase (Cai *et al.*, 2022). Macrosporusone D is a polyketide isolated from marine-derived fungi has also demonstrated antibacterial effects against Gram-positive bacterium *Staphylococcus aureus* with an MIC of 6.25µg/mL (Song *et al.*, 2022). These compounds highlight the relevance of *Talaromyces* marine fungi in the treatment of several diseases and in drug devellopement.

2 Bacteria

2.1 *Streptomyces sp*

2.1.1 Taxonomy

Kingdom	***Bacillati***
Phylum	***Actinomycetota***
Class	***Actinomycetes***
Order	***Kitasatosporales***
Family	***Streptomycetaceae***
Genus	***Streptomyces***

2.1.2 Geographical distribution and morphology

Recent studies have indicated that several *Actinomycetota* taxa derived from marine environment are a prolific source within their high genetic capacities in producing large

amounts of antimicrobial, antiviral, immunosuppressant and anticancer natural compounds which are significantly important in pharmaceuticals and industrial discoveries (Nouioui *et al.*, 2025). In particular, the genus *Streptomyces* have been observed in different extreme physiological conditions in marine habitats with a high-pressure environment, pH, temperature, salinity, and light intensity (Shi *et al.*, 2022).

Streptomyces sp. has generally been recorded in diverse marine ecosystems, such as west-central in Philippines (Tenebro *et al.*, 2021), the Andaman coast in the eastern Indian Ocean (Gopalakrishnan *et al.*, 2014), the central Cantabrian Sea the north coast of Spain (Braña *et al.*, 2015), the Oker River, Braunschweig, Germany (Nouioui *et al.*, 2025), mangrove sediment in Bali Island, Indonesia (Damayanti *et al.*, 2021), the Ras Garib area of the Gulf of Suez (Hamed *et al.*, 2021) and Mersa Matruth City located on the northern coast of the Mediterranean Sea in Egypt (Kim *et al.*, 2021).

Although *Streptomyces sp*. is particularly prevalent in these regions, it is important to note that *Streptomyces althioticus* may exhibit a similar or overlapping distribution. The marine *Streptomyces sp*. strains produce colonies with well-developed white (Figure 14) aerial mycelium after five days of cultivation on Gauze 2 medium. This mycelium can be easily removed with a loop. On Eshby's medium, the aerial mycelium appears cream-coloured. The colonies produce a diffusible, dark brown, water-soluble pigment in both media, with the most active production occurring in TSB liquid nutrient medium (Гудзенко *et al.*, 2023).

Morphological examination under scanning electron microscopy reveals sporophores bearing oval-shaped spores with smooth surfaces (Lu *et al.*, 2009). In particular, *Streptomyces althioticus* produced black, round-haired spores which form chains had brown pigmentation (Figure 14).

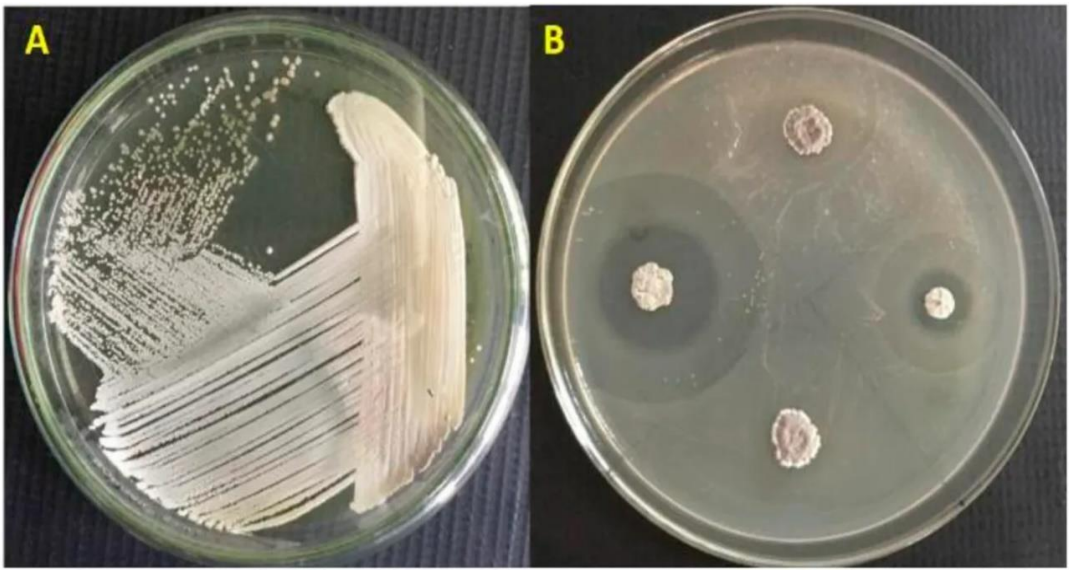


Figure 14: (A) Colony morphology of *Streptomyces sp*. VITGV156, (B) Antibacterial screening revealed that VITGV156 exhibited inhibition E. coli (Pattapulavar *et al.*, 2025).

2.1.3 Derived metabolites

Marine *streptomycetes* are the main producers of various secondary metabolites with high biological activity, making them an important source of new antibiotics, anti-inflammatory agents, anticancer agents, and more. For example, 1-hydroxy-1-norresistomycin, a polyketide isolated from this genus, exhibited significant cytotoxicity against various blood cancer cell lines and moderate activity against solid cancer cell lines, with GI_{50} values ranging from 0.33 to 1.24 µM and 6.67-10.16 µM, respectively (Shin *et al.*, 2026).

In addition, *Wenwen Yi et al*., reported that hygrocins N and other compounds collected from *Streptomyces sp.* demonstrated potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*, with minimum inhibitory concentrations (MIC) values ranging from 3 to 48 µg/mL (Yi *et al.*, 2022). Moreover, chlororesistoflavins A and B were obtained from a marine-derived *Streptomyces* strain. Chlororesistoflavin A exhibited antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) that was comparable to that of resistoflavin (MIC = 0.25 µg/mL) and eight times more potent than that of chlororesistoflavin B, which had an MIC of 2.0 µg/mL (Wibowo *et al.*, 2023).

MATERIALS AND METHODS

1. Databases and webservers

1.1. Protein Data Bank (PDB)

The Protein Data Bank (https://www.rcsb.org/), established in 1971, is recognised as the first digital data resource in biology with free access (Figure 15). It contains more than 254,186 experimentally determined three-dimensional (3D) biomolecular structure data, including proteins, nucleic acids (DNA and RNA), and their complexes with one another and with small-molecule ligands. Each year, tens of thousands of macromolecular structure data are received from various experimental techniques, such as macromolecular crystallography, nuclear magnetic resonance spectroscopy, electron microscopy and micro-electron diffraction **(Burley *et al.*, 2022)**. The PDB provides different tools and services to help the scientific community and the public access, visualise and understand 3D biomolecular structure data **(Burley *et al.*, 2022)**.

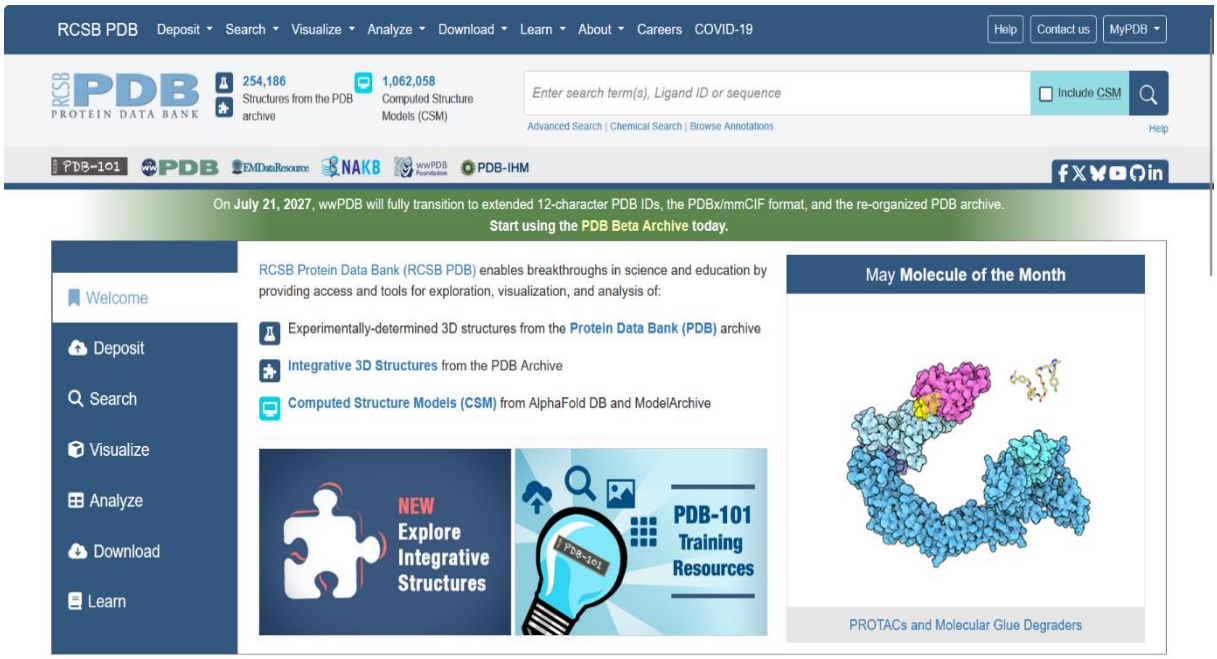


Figure 15: Recent screenshot of the RCSB PDB homepage, accessed on May 20, 2026.

1.2. Pubchem

PubChem (https://pubchem.ncbi.nlm.nih.gov) is an accessible and free database that offers a global view of available information on chemical structures and biological activities of various

molecules, it is developed by NCBI (Figure 16). Over the last two years, the platform has been visited by a large number of users worldwide each month, and currently has undergone principal updates, especially the additions from more than 130 new sources (Kim *et al.*, 2025).

PubChem data are arranged into several collections:

- **Substance**: Data providers supplied chemical information to PubChem.
- **Compound**: Unique chemical structures obtained from recorded substance in PubChem.
- **BioAssays**: contributors distributed biological experimental data of molecules.
- **Protein and gene**: protein and gene used in PubChem pathway and bioassay experiments.
- **Pathway**: Cellular changes caused by the interactions among sets of chemicals, genes, and proteins.
- **Cell line and taxonomy**: Cells and organisms of tested molecules evaluated in experiments.
- **Patent**: Patent information in PubChem.

PubChem provides a comprehensive data about the safety, environmental effects, chemical structure and biological activities of target compounds, which can assist researchers in their studies and discoveries (Kim *et al.*, 2025).

Figure 16: Recent screenshot of the PubChem database homepage, accessed on May 20, 2026.

1.3. Pre-ADMET

PreADMET (https://preadmet.webservice.bmdrc.org/toxicity/) serves (Figure 17) as an initial prediction tool for the pharmacokinetic and toxicological characteristics of small molecules, providing free access to important ADMT properties (Absorption, Distribution, Metabolism, and Toxicity). This helps scientists estimate the drug-likeness and safety profile of chemical compounds at an early stage of compound screening, before laboratory testing (Dulsat *et al.*, 2023). The server accepts entries in various formats, including SMILES strings and molecular structure files (like MOL or SDF), and features a user-friendly interface for analysing and entering multiple structures (Dulsat *et al.*, 2023).

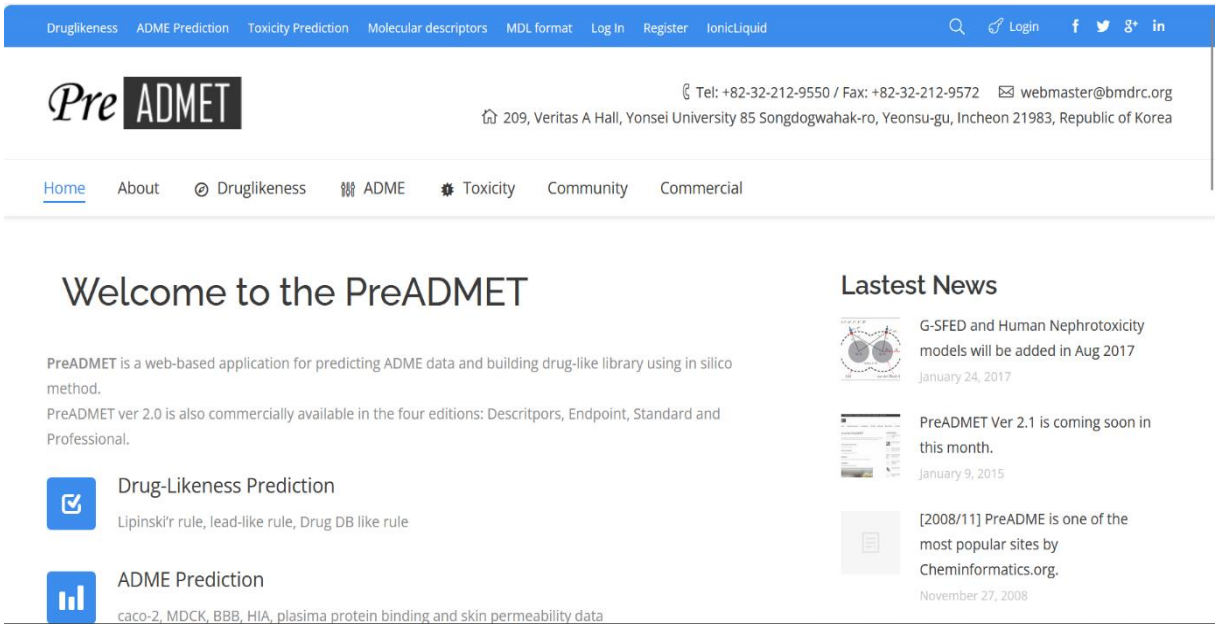


Figure 17: Screenshot of the PreADMET server homepage, accessed on May 20, 2026.

2. Software's and programs

2.1. UCSF chimera

UCSF Chimera v1.19 is a molecular modeling and visualization program widely used for the analysis of biomolecular structures (Butt *et al.*, 2020). In this study, UCSF Chimera was used as the platform for conducting molecular docking and analyzing the resulting complexes. The software provides an integrated environment for the preparation, visualization, and evaluation of biomolecular structures, which facilitates efficient docking studies. Protein structures

obtained from the Protein Data Bank were imported into UCSF Chimera, where the program enabled convenient structural inspection and preparation prior to computational analysis. Its advanced visualization capabilities allowed clear examination of ligand-protein binding conformations and interactions, supporting the interpretation of docking outcomes and the generation of high-quality molecular representations for analysis and presentation.

2.2. Discovery studio visualizer

Discovery studio visualizer (DSV) v21 is a free software tool widely used for analyzing, viewing and modeling molecular structures (Prajapati *et al.*, 2022). It provides a group of data regarding 3D molecular structures (Figure 18) by mentioning all the important protein-ligand interactions, notably hydrogen and hydrophobic bonds which participate on the stability and affinity of divers structures (Prajapati *et al.*, 2022). Other main and useful features available in this program is a high-resolution graphic rendering, electrostatic potential mapping and graphical adjustment setting, that help researchers to modify several options such as residue and background color, appearance, size and labels (Prajapati *et al.*, 2022).

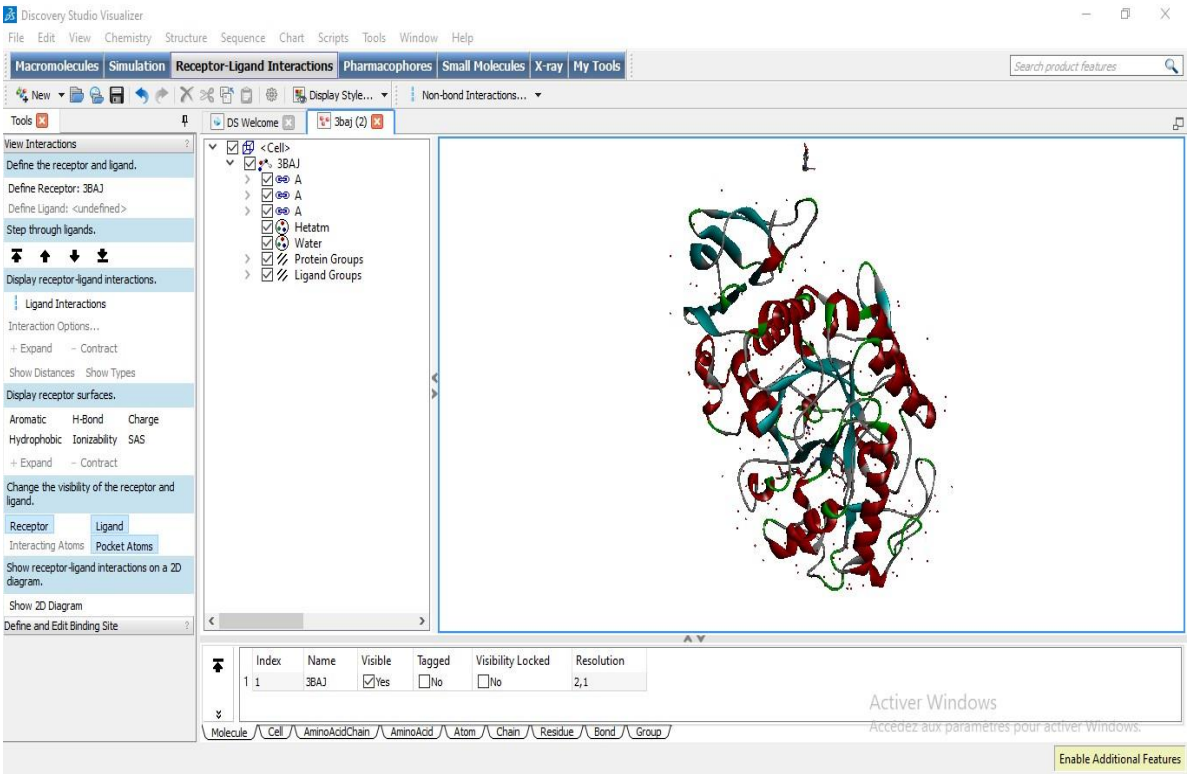


Figure 18: The primary interface of the DSV software.

2.3.ChemDraw professional

ChemDraw professional v16.0 (Figure 19) 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 (Brown, 2014).

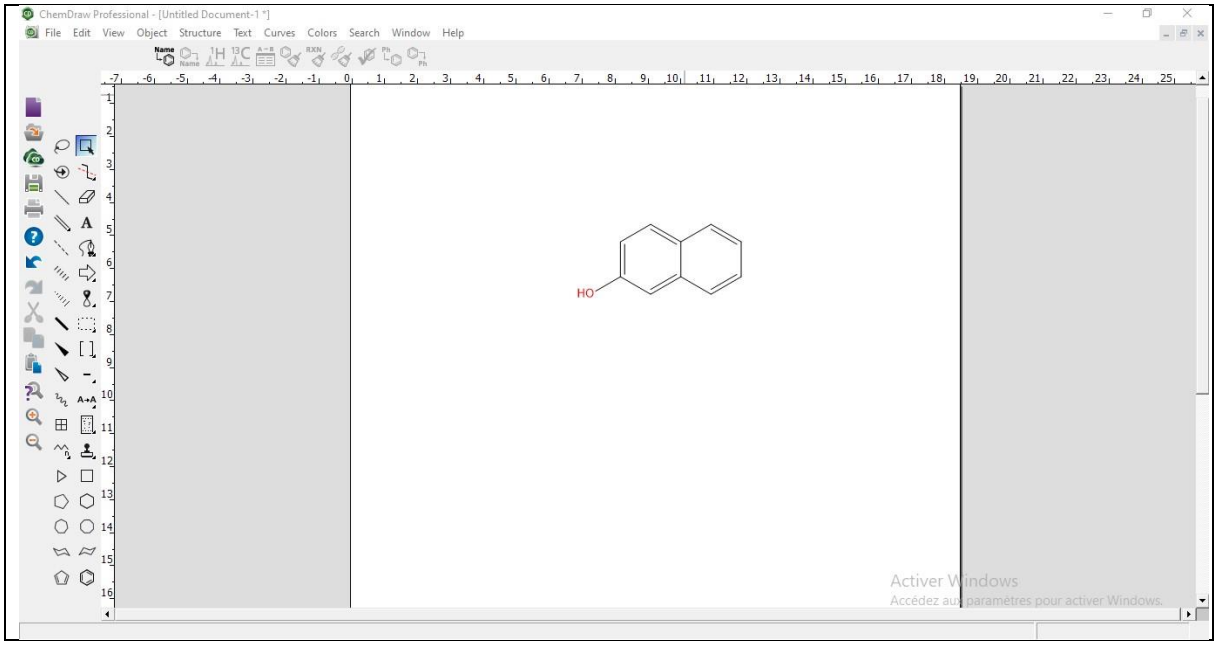


Figure 19: Chemdraw program interface.

3. Absorption, Distribution, Metabolism and Toxicity

The high drug development failure rate, estimated at nearly 90% in recent decades, is largely attributed to insufficient prediction of drug behavior within biological systems, which can affect safety and increase toxicity risks in living organisms. Therefore, the evaluation of the ADMET profile including Absorption, Distribution, Metabolism, Excretion, and Toxicity (Figure 20) plays a crucial role in improving the success rate of drug discovery and development processes (Dulsat *et al.*, 2023). The ADMT proprieties reflect the absorption mechanism of the molecules

into blood circulation, diffusion throughout several tissues and organs sites, metabolic conversion catalyzed by hepatic enzymes, and the potential toxicity or harmful effects induced by these compounds. This predictive tool enables researchers to reduce time consuming, financial costs and minimize the risk of late-stage experimental failure, by highlighting promising results and select suitable candidates with favorable ADMT profiles, prior to *in vitro* or *in vivo* assays (Fan *et al.*, 2025). In this study, we collected information from PreADMET v2.0 server to identify the ADMT proprieties and compare our selected molecules with Acarbose as the reference drug.

The excretion properties of the investigated compounds were not assessed in this study because the required predictive data for this parameter were unavailable.

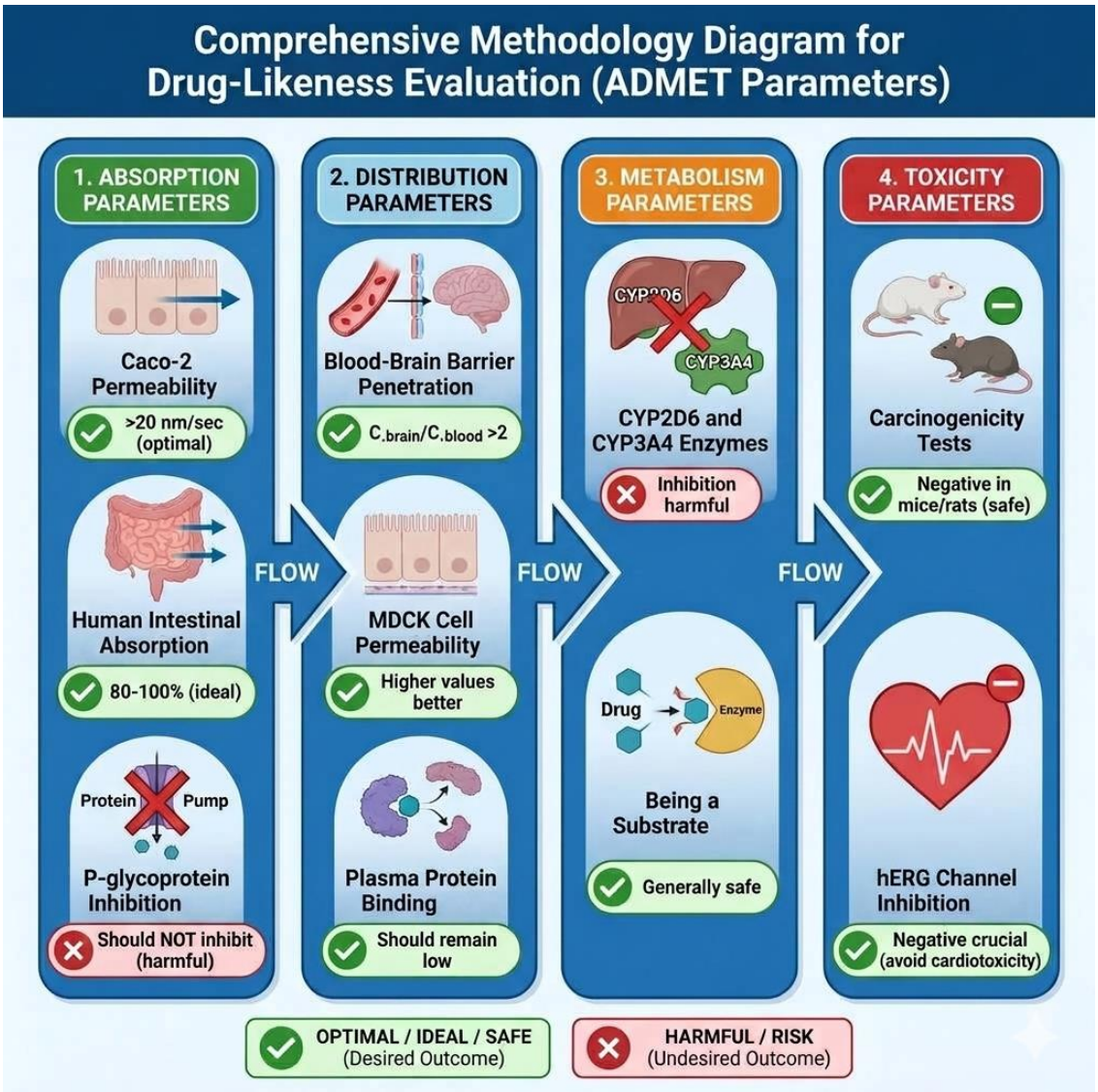


Figure 20: Parameters used in the ADMT study (Amin, 2013; Jing *et al.*, 2015; Kratochwil *et al.*, 2002; Lynch & Price, 2007; van der Laan *et al.*, 2016; Volpe, 2011; Waters *et al.*, 2016).

4. Molecular docking

Molecular docking (MD) is a computational approach used to predict molecular interactions between small molecules and target proteins or enzymes (Anusha & Kamala, 2025). It is widely applied in drug discovery to evaluate ligand binding strength and determine the spatial orientation of compounds within the receptor's active site (Fan *et al.*, 2019). In this study, MD was performed to evaluate the binding affinity of 23 selected marine-derived compounds toward human pancreatic α-amylase (HPA).

MD was carried out using AutoDock Vina implemented through UCSF Chimera (Butt *et al.*, 2020). The three-dimensional structures of the ligands were obtained from the PubChem database (Table 1) in SDF format and prepared using the default geometry optimization procedures in Chimera.

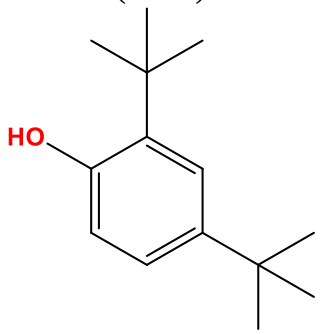
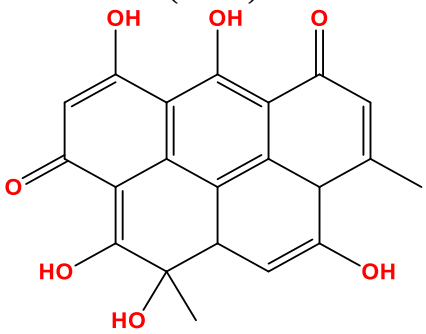
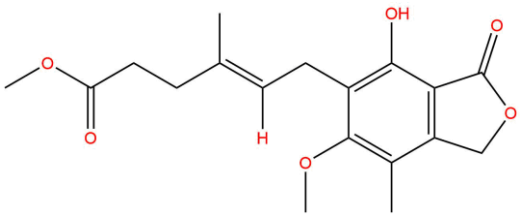
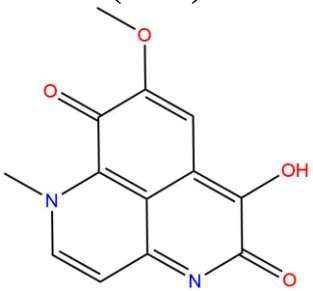
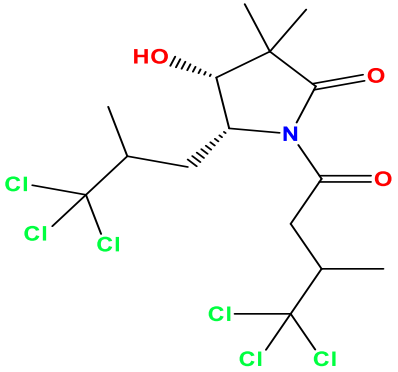
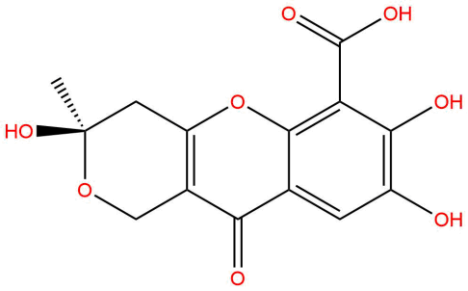
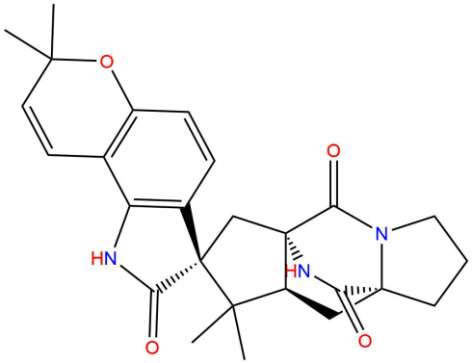
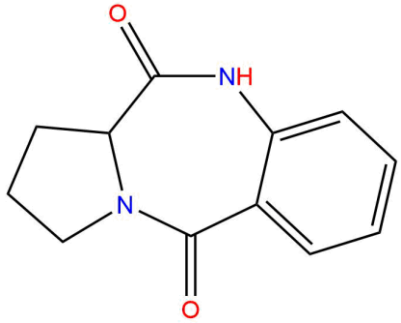
Initially, the crystal structure of human pancreatic α-amylase (PDB ID: 3BAJ) was retrieved from the Protein Data Bank. The structure contains a single polypeptide chain (A) composed of 496 amino acids and includes three heteroatoms: 2-acetamido-2-deoxy-β-D-glucopyranose (NAG), nitrate ion (NO_3^-), and calcium ion (Ca^{2+}). The enzyme was co-crystallized with Acarbose, which was used as a positive control for docking validation. Protein preparation involved several steps.

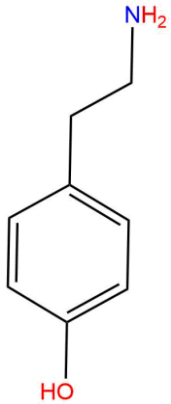
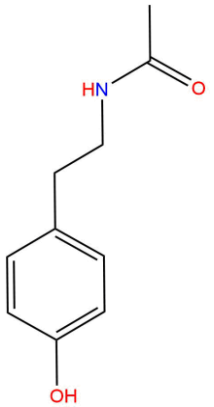
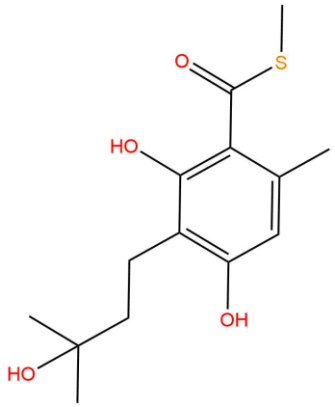
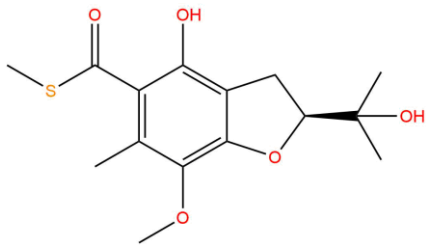
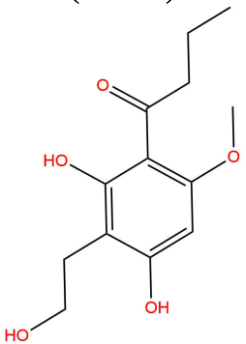
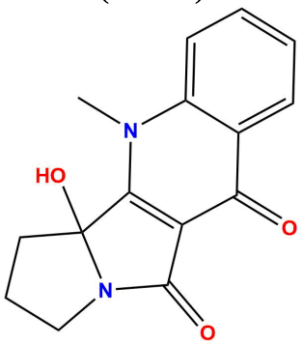
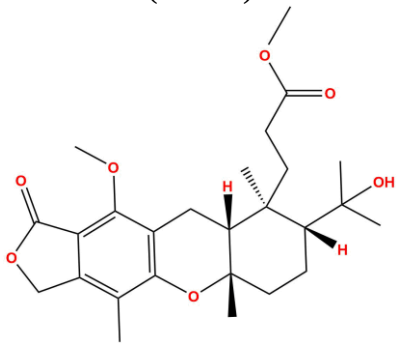
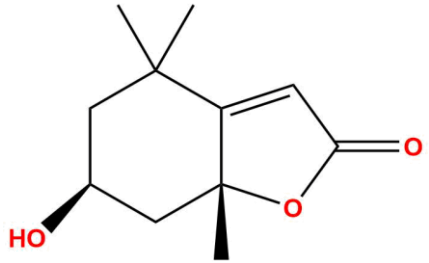
- First, water molecules, unnecessary heteroatoms, and co-ligands were removed while preserving essential components within the active site.
- Second, polar hydrogens and partial charges were added to the protein.
- Third, a grid box was defined to specify the docking search space.

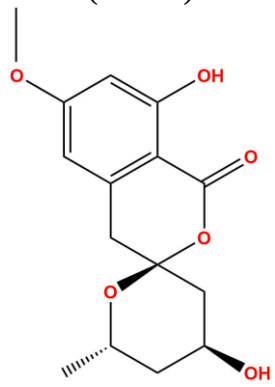
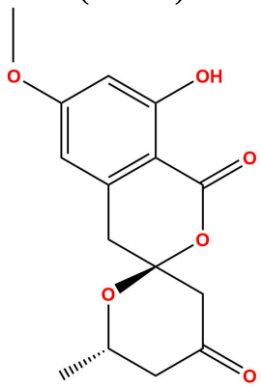
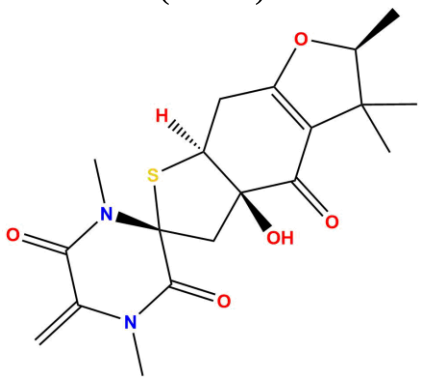
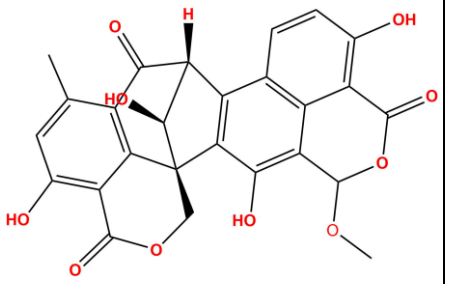
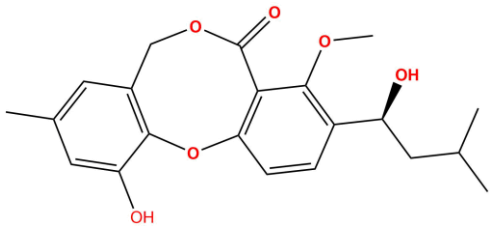
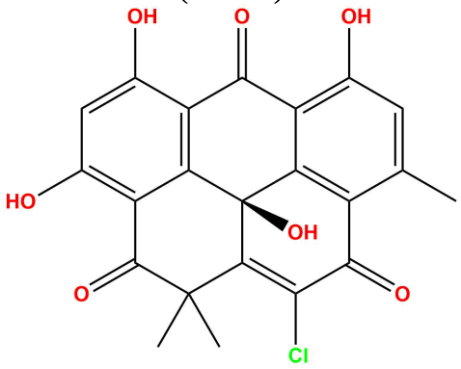
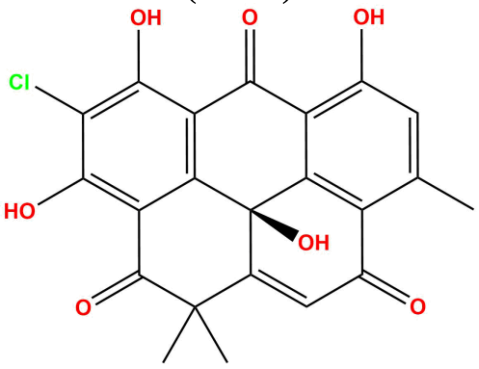
Two docking strategies were applied: blind docking and focused docking. Blind docking was first conducted to explore the entire protein surface and identify potential ligand binding regions without prior assumptions. Subsequently, focused docking was performed by centering the grid

box on the catalytic site of HPA with center coordinates (x = 9.611, y = 17.023, z = 41.176) and dimensions (x = 22, y = 16, z = 18) to obtain more accurate predictions of ligand-target interactions. For each ligand, multiple binding poses were generated, and the best conformations were selected based on their binding affinity (kcal/mol) and interactions with key residues within the catalytic site. The docking results were further analyzed using DSV to identify the most promising compounds showing stronger binding affinity compared with the reference inhibitor Acarbose.

Table 1: Used compounds 2D structures and their major chemical properties.

2,4-Di-tert-butylphenol (Mol1)  MF: $C_{14}H_{22}O$ MW: 206.32 g/mol MC: Phenols Prod. org: *Marine Streptomyces* PubChem ID: 7311 Source: (Patel *et al.*, 2026)	1-hydroxy-1-norresistomycin (Mol2)  MF: $C_{21}H_{14}O_7$ MW: 378.3 g/mol MC: Polyketides Prod. org: *Streptomyces althioticus* PubChem ID: 135467113 Source: (Shin *et al.*, 2026)	Mycophenolic acid methyl ester (Mol3)  MF: $C_{18}H_{22}O_6$ MW: 334.4 g/mol MC: Polyketides Prod. org: *Phaeosphaer-ia spartinae* PubChem ID: 6478685 Source: (Zihan Wang *et al.*, 2025)	Aaptanone (Mol4)  MF: $C_{13}H_{10}N_2O_4$ MW: 258.23 g/mol MC: Alkaloids Prod. Org: *Aaptos lobata* PubChem ID: 135879380 Source: (Makinde *et al.*, 2025)
Dysidamide (Mol5)  MF: $C_{15}H_{21}Cl_6NO_3$ MW: 476.0 g/mol MC: Alkaloids Prod. org: *Spongia sp* PubChem ID: 6711373 Source: (Tai *et al.*, 2022)	Penialidin A (Mol6)  MF: $C_{14}H_{12}O_8$ MW: 308.24 g/mol MC: Polyketides Prod. org: *Penicillium sp.* PubChem ID: 139584805 Source: (Zimin Wang, Meirong Zhao, Yunxia Yu, *et al.*, 2025)	Notoamide B (Mol7)  MF: $C_{26}H_{29}N_3O_4$ MW: 447.5 g/mol MC: Alkaloid Prod. org: *Aspergillus sclerotiorum* PubChem ID: 16127923 Source: (Mao *et al.*, 2023)	Cycloanthranilylproline (Mol8)  MF: $C_{12}H_{12}N_2O_2$ MW: 216.24 g/mol MC: Alkaloids Prod. org: *Metarhizium sp.* PubChem ID: 551139 Source: (Yao *et al.*, 2022)

Tyramine (Mol9)  MF: $C_8H_{11}NO$ MW: 137.18 g/mol MC: Alkaloids Prod. org: *Shewanella aquimarina* PubChem ID: 5610 Source: (Giugliano *et al.*, 2023)	N-(4-hydroxyphenethyl) acetamide (Mol10)  MF: $C_{10}H_{13}NO_2$ MW: 179.22 g/mol MC: Alkaloids Prod. org: *Shewanella aquimarina* PubChem ID: 121051 Source: (Giugliano *et al.*, 2023)	Eurothiocin G (Mol11)  MF: $C_{14}H_{20}O_4S$ MW: 284.37 g/mol MC: Polyketides Prod. org: *Talaromyces indigoticus* PubChem ID: 170990184 Source: (Li *et al.*, 2021)	Eurothiocin C (Mol12)  MF: $C_{15}H_{20}O_5S$ MW: 312.4 g/mol MC: Polyketides Prod. org: *Talaromyces indigoticus* PubChem ID: 170990180 Source: (Li *et al.*, 2021)
Phomalone (Mol13)  MF: $C_{13}H_{18}O_5$ MW: 254.28 g/mol MC: Aromatic ketone Prod. org: *Alternaria sp.* PubChem ID: 178026 Source: (Zhong *et al.*, 2021)	Oxypenicinoline A (Mol14)  MF: $C_{15}H_{14}N_2O_3$ MW: 270.28 g/mol MC: Alkaloids Prod. org: *Penicillium steckii* PubChem ID: 170988835 Source: (Zimin Wang, Meirong Zhao, Yunxia Yu, *et al.*, 2025)	Austalide P (Mol15)  MF: $C_{26}H_{36}O_7$ MW: 460.6 g/mol MC: Terpenoids Prod. org: *Penicillium thomii* PubChem ID: 56839853 Source: (Zimin Wang, Meirong Zhao, Yunxia Yu, *et al.*, 2025)	Loliolide (Mol16)  MF: $C_{11}H_{16}O_3$ MW: 196.24 g/mol MC: Terpenoids Prod. org :*Bifurcaria bifurcata* PubChem ID : 100332 Source: (Veríssimo *et al.*, 2021)

Penicillol A (Mol17)  MF: $C_{15}H_{18}O_6$ MW: 294.30 g/mol MC: Polyketide Prod. org: *Penicillium sp.* PubChem ID : 170990095 Source: (Chen *et al.*, 2022)	Penicillol B (Mol18)  MF: $C_{15}H_{16}O_6$ MW: 292.28 g/mol MC: Polyketide Prod. org: *Penicillium sp.* PubChem ID : 170990096 Source: (Chen *et al.*, 2022)	Talaromanloid A (Mol19)  MF: $C_{19}H_{24}N_2O_5S$ MW: 392.5 g/mol MC: alkaloid Prod. org: *Talaromyces mangshanicus* PubChem ID : 170990212 Source: (Zhang *et al.*, 2022)	Macrosporusone D (Mol20)  MF: $C_{26}H_{18}O_{10}$ MW: 490.4 g/mol MC: Polyketide Prod. org: *Talaromyces sp* PubChem ID: 146683386 Source: (Song *et al.*, 2022)
Penicillide (Mol21)  MF: $C_{21}H_{24}O_6$ MW: 372.4 g/mol MC: Polyketide Prod. org: *Talaromyces sp* PubChem ID: 124213 Source: (Song *et al.*, 2022)	Chlororesistoflavin A (Mol22)  MF: $C_{22}H_{15}ClO_7$ MW: 426.8 g/mol MC: Polyketide Prod. org: *Streptomyces sp.* PubChem ID: 168294906 Source: (Wibowo *et al.*, 2023)	Chlororesistoflavin B (Mol23)  MF: $C_{22}H_{15}ClO_7$ MW: 426.8 g/mol MC: Polyketide Prod. org: *Streptomyces sp.* PubChem ID: 168280684 Source: (Wibowo *et al.*, 2023)	**MF**: Molecular formula **MW**: Molecular weight **MC**: Metabolite class **Prod**. Org: Producer organism

RESULTS AND DISCUSSION

1. Absorption, Distribution, Metabolism and Toxicity

The predicted ADMET parameters for the 23 selected compounds are summarized in Table 2. The results indicate variable absorption properties among the investigated molecules. Compounds Mol1, Mol2, Mol4, Mol6, Mol7, Mol8, Mol11, Mol12, Mol14, Mol15, Mol16, Mol20, and Mol22 exhibit high Caco-2 cell permeability values (>20 nm/sec), suggesting strong ability to cross intestinal epithelial barriers and favorable membrane penetration. In contrast, Mol10 and Mol13 shows very low permeability (<2 nm/sec), indicating limited intestinal transport capacity. The remaining compounds, including Mol3, Mol5, Mol9, Mol17, Mol18, Mol19, Mol21, Mol23, and the control compound, present moderate permeability values ranging from approximately 8 to 20 nm/sec, suggesting moderate intestinal diffusion potential.

Despite the differences observed in Caco-2 permeability, most of the compounds demonstrate high predicted human intestinal absorption (HIA), with values ranging from approximately 80 % to 97 %, indicating generally favorable oral absorption characteristics. These results suggest that several compounds may still achieve effective gastrointestinal uptake even when their passive permeability is moderate.

Regarding P-glycoprotein (P-gp) interactions, the majority of the compounds, including Mol12, Mol13, Mol14, Mol16, Mol17, Mol18, Mol19, Mol20, and Mol21, are predicted to be non-inhibitors of P-gp, suggesting a reduced likelihood of active efflux and improved intracellular retention. In contrast, Mol1, Mol3, Mol5, Mol11, Mol15, Mol22, Mol23, and the control compound are predicted to act as P-gp inhibitors, which may potentially affect the pharmacokinetics of co-administered drugs and increase the possibility of drug-drug interactions.

The predicted distribution profiles reveal limited blood-brain barrier (BBB) permeability for all investigated compounds except Mol1, with values ranging between approximately 0.02 and

0.76. Since BBB penetration generally requires values greater than 2, these results indicate that the studied molecules are unlikely to efficiently cross the BBB, suggesting minimal central nervous system exposure.

MDCK cell permeability, which serves as a model for tissue distribution, shows notable variability among the compounds. Mol1, Mol9, Mol10, Mol11, Mol12, Mol13, Mol16, Mol17, and Mol18 demonstrate high permeability values (>10 nm/sec), indicating strong potential for effective tissue distribution. Mol2, Mol4, Mol7, Mol8, Mol14, Mol22, and Mol23 display moderate permeability values between 1 and 10 nm/sec, suggesting moderate tissue penetration. In contrast, Mol3, Mol5, Mol6, Mol15, Mol19, Mol20, Mol21, and the control compound exhibit very low MDCK permeability (<2.5 nm/sec), which may limit their systemic distribution and tissue absorption.

The predicted plasma protein binding (PPB) values reveal different binding affinities among the compounds. Mol1, Mol2, Mol3, Mol5, Mol11, Mol12, Mol13, Mol15, Mol20, Mol21, Mol22, and Mol23 show high plasma protein binding (≥80 %), indicating strong interaction with plasma proteins such as albumin. High PPB may prolong systemic circulation but can reduce the fraction of free pharmacologically active drug. Conversely, Mol6, Mol7, Mol8, Mol14, Mol17, and Mol18 display moderate binding (40-80 %), while Mol4, Mol9, Mol10, Mol16 Mol19 and the control exhibit relatively low PPB values (<40 %), suggesting a higher proportion of free drug available in circulation and potentially improved immediate bioavailability.

Cytochrome P450 metabolism predictions provide insights into potential metabolic interactions. None of the investigated compounds are predicted to inhibit CYP2D6, except for Mol9 and the control compound, indicating generally low metabolic interference through this enzyme pathway. Regarding CYP3A4 inhibition, several compounds and the control compound are predicted to act as inhibitors, suggesting potential interactions with drugs metabolized

through this major hepatic isoenzyme. In contrast, Mol4, Mol5, Mol8, Mol9, Mol14, Mol17, Mol19, and Mol21 predicted none inhibitors toward CYP3A4, indicating a potentially more favorable metabolic profile with reduced risk of enzyme-mediated interactions.

Toxicity predictions demonstrate encouraging safety profiles for the investigated compounds. It is important to note that toxicity assessment constituted one of the primary filtering criteria during the compound selection process. From the beginning of the study, only molecules predicted to exhibit acceptable safety profiles were retained for further pharmacokinetic and molecular analyses. Consequently, all selected compounds are predicted to be non-carcinogenic in both mouse and rat models, indicating a low potential for carcinogenic effects. Furthermore, all compounds display a low risk of hERG channel inhibition, suggesting minimal likelihood of cardiotoxicity. In contrast, the control compound shows an ambiguous risk of hERG inhibition, which may indicate a comparatively higher concern regarding potential cardiac safety compared with the screened molecules.

Table 2: ADMT parameters of the selected compounds (green = low risk, orange = medium risk, red = high risk).

Pharmacokinetics	Mol1	Mol2	Mol3	Mol4	Mol5	Mol6	Mol7	Mol8	Mol9	Mol10	Mol11	Control
Absorption												
Caco-2 cell permeability (nm/sec) > 20	44.86	21.02	18.87	20.01	19.99	20.78	21.41	21.04	19.35	1.80	20.41	8.17
Human intestinal absorption (HIA %) 80 to 100 %	100	83.62	95.47	96.61	97.09	47.85	94.3	94.77	90.48	89.20	87.50	0
P-glyco protein inhibition should not inhibit a substrate of it indicates high levels absorption	Inhi	Non	Inhi	Non	Inhi	Non	Non	Non	Non	Non	Inhi	Inhi
Distribution												
Blood-brain barrier penetration (C.brain/C.blood) (BBB) > 2 cross the blood–brain barrier easily	10.09	0.149	0.040	0.08	3.73	0.24	0.04	0.52	0.62	0.80	1.15	0.027
MDCK cell permeability (nm/sec) Low (< 1 nm/s), moderate (1-10 nm/s), and high (>10 nm/s)	116.431	2.68	0.098	2.154	0.053	0.89	2.22	6.40	28.5	23.56	32.9	0.516
Plasma protein binding (%) (PPB) 80 to 100 % is considered high, 50 to 80 % (moderate), < 50 % (low)	100	81.66	87.65	30.76	93.99	53.80	66.96	40.26	0.45	35.6	95.73	0
Metabolism												
Cytochrome P450 2D6 inhibition	Non	Non	Non	Non	Non	Non	Non	Non	Inhi	Non	Non	Inhi
Cytochrome P450 2D6 substrate	Non	Non	Non	Non	Non	Non	Non	Non	Sub	Weakly	Non	Weakly
Cytochrome P450 3A4 inhibition	Inhi	Inhi	Inhi	Non	Non	Inhi	Inhi	Non	Non	Inhi	Inhi	Inhi
Cytochrome P450 3A4 substrate	Sub	Sub	Sub	Sub	Sub	Weakly	Sub	Non	Weakly	Non	Sub	Sub
Toxicity												
Carcinogenicity (Mouse)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Carcinogenicity (Rat)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
HERG_inhibition risk	low	low	low	low	low	low	low	low	low	low	low	Ambig

HERG**: Human ether related gene channel** MDCK**: Mandin Darby Canine Kidney** Caco-2**: Human colorectal carcinoma** Inhi**: Inhibitor** Sub**: Substrate** Neg: **Negative** Ambig**: Ambiguous**

Pharmacokinetics	Mol12	Mol13	Mol14	Mol15	Mol16	Mol17	Mol18	Mol19	Mol20	Mol21	Mol22	Mol23	Control
Absorption													
Caco-2 cell permeability (nm/sec) > 20	20.33	0.73	21.3	27.6	21.5	19.2	19.8	18.2	20.6	9.52	20.63	19.12	8.17
Human intestinal absorption (HIA %) 80 to 100 %	92.24	80.37	95.8	97.21	93.37	88.78	92.2	95.25	86.76	94.02	87.81	87.8	0
P-glyco protein inhibition should not inhibit a substrate of it indicates high levels absorption	Non	Non	Non	Inhi	Non	Non	Non	Non	Non	Non	Inhi	Inhi	Inhi
Distribution													
Blood-brain barrier penetration (C.brain/C.blood) (BBB) > 2 cross the blood–brain barrier easily	0.76	0.76	0.57	0.03	0.50	0.50	0.55	0.11	0.02	0.75	0.53	0.65	0.027
MDCK cell permeability (nm/sec) Low (< 1 nm/s), moderate (1-10 nm/s), and high (>10 nm/s)	105.6	47.33	4.14	0.063	36.6	12.0	12.02	0.62	2.31	0.06	3.90	6.29	0.516
Plasma protein binding (%) (PPB) 80 to 100 % is considered high, 50 to 80 % (moderate), < 50 % (low)	80.43	81.20	56.65	87.6	23.88	67.32	69.90	43.50	81.33	90.84	96.42	93.27	0
Metabolism													
Cytochrome P450 2D6 inhibition	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Inhi
Cytochrome P450 2D6 substrate	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Weakly
Cytochrome P450 3A4 inhibition	Inhi	Inhi	Non	Inhi	Inhi	Non	Inhi	Non	Inhi	Non	Inhi	Inhi	Inhi
Cytochrome P450 3A4 substrate	Sub	Non	Non	Sub	Weakly	Weakly	Sub	Sub	Sub	Sub	Sub	Sub	Sub
Toxicity													
Carcinogenicity (Mouse)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Carcinogenicity (Rat)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
HERG_inhibition risk	low	low	low	low	low	low	low	low	low	low	low	low	ambig

HERG**: Human ether related gene channel** MDCK**: Mandin Darby Canine Kidney** Caco-2**: Human colorectal carcinoma** Inhi**: Inhibitor** Sub**: Substrate** Neg: **Negative** Ambig**: Ambiguous**

2. Molecular docking

The results obtained following focused docking are presented in Table 3, which is based on a comparison of the binding energy with that of the control, acarbose, while blind docking results were excluded since they matched the focused docking outcomes. The energy of the 12 molecules ranged from -8.0 to -10.0 kcal/mol, with Mol7 (notoamide B) exhibiting the best score of -10.0 kcal/mol compared to the control value of -7.9 kcal/mol.

Table 3: Analysis of the MD results.

Ligand	Energy (Kcal/mol)	Active site residues	Hydrophobic Electrostatic/ Interactions	Hydrogen bond (Å)	Fav /Unfav bond
Acarbose (Control)	**-7.9**	TRP58, TYR62 GLN63, THR163 ASP197, GLU233 ASP300, HIS305	π-Alkyl	GLN63: 3.08 THR163: 2.31 TYR62: 2.84 GLU233: 2.87 HIS305: 2.44 ASP197: 3.02	9/2
1-hydroxy-1-norresistomycin (mol2)	**-9.2**	TRP58, TRP59, GLN63, HIS305	π-π Stacked π -Alkyl	GLN63(2.68)	11/0
Penialidin A (Mol6)	**-8.3**	TRP59, TYR62, THR163, LEU165, ASP197, ALA198, GLU233, ASP300, HIS305	π -Sigma π-π T shaped Alkyl π -Alkyl	HIS305(2.19) GLU233(2.81) ASP300(2.19) ASP197(1.98) THR163(2.71)	10/0
Notoamide B (Mol7)	**-10.0**	TRP59, HIS101, TYR151, LEU162, LEU165, HIS201, ILE235, ASP300, GLY306	Alkyl π-Alkyl	ASP300(1.93) GLY306(3.00)	9/0
Oxypenicinoline A (Mol14)	**-8.0**	TRP58, TRP59, TYR62, ASP197, HIS305	π-π Stacked π-Alkyl	ASP197(3.51)	7/0
Austalide P (Mol15)	**-8.1**	TRP58, TRP59, TYR62, HIS101, LEU162, LEU165 ALA198, ASP300	π-Sigma Alkyl π-Alkyl π-Anion	-	13/0
Penicillol A (Mol17)	**-8.1**	TRP59, GLN63 LEU162, LEU165 ASP300	π-π Stacked Alkyl π-Alkyl	GLN63(2.18) ASP300(2.96)	8/0
Penicillol B (Mol18)	**-8.5**	TYR62, LEU162 LEU165, ARG195 ALA198, ASP300	π-π Stacked Alkyl	ARG195 (2.03) ASP300 (1.96)	6/0
Talaromanloid A (mol19)	**-9.4**	TYR151, LEU162 LEU165, ASP197	Alkyl π-alkyl	GLU233 (2.28)	10/1

		ALA198, HIS201 GLU233, ILE235 ASP300		TYR151 (3.55) ASP197 (2.93)	
Macrosporusone D (mol20)	**-9.0**	HIS101, LEU162 LEU165, ALA198 HIS305	π –alkyl Alkyl	HIS305 (2.27)	6/0
Penicillide (mol21)	**-8.5**	LEU165, HIS201 GLU233, ILE235 ASP300	π-Sigma Alkyl π-Alkyl π-Anion	ASP300 (2.66)	6/1
Chlororesistoflavins A (mol22)	**-9.5**	TRP59, TYR62 LEU162, THR163 LEU165, ASP197 ALA198	π-Sigma π-Alkyl	THR163 (2.28) ASP197 (2.01) TYR62 (2.58)	8/0
Chlororesistoflavin B (mol23)	**-9.5**	TRP59, LEU165 ASP197, GLU233 ASP300	π-Sigma π-Alkyl π-Anion	ASP197 (2.88) TRP59 (2.62)	7/0

Å : Angstrom **Fav /Unfav:** Favorable/Unfavorable

α-Amylase (EC 3.2.1.1) is a calcium-dependent metalloenzyme secreted primarily by the salivary glands and the pancreas. This enzyme catalyzes the hydrolysis of internal α-1,4-glycosidic linkages in complex carbohydrates such as starch and glycogen, producing maltose, dextrins, and various oligosaccharides that can subsequently be converted into glucose monomers during digestion. In addition to animals, α-amylase is naturally synthesized by plants and microorganisms, reflecting its widespread biological importance (Kaur *et al.*, 2021; Shad *et al.*, 2023).

The crystal structure of the enzyme was reported in complex with the reference inhibitor acarbose. Docking validation confirmed that acarbose occupies the catalytic pocket of α-amylase (Table 4), forming several conventional hydrogen bonds with key residues including GLU233, HIS305, GLN63, THR163, and TYR62. A carbon-hydrogen bond interaction was also observed with ASP197, along with a π-alkyl hydrophobic interaction. Two unfavorable contacts with ASP300 and THR163 were detected. The predicted binding energy of the acarbose-enzyme complex was -7.9 kcal/mol. The 3D structures of the 12 docked complexes are presented in Table 4.

Mol7, identified as notoamide B, is an indole alkaloid that exhibited the lowest binding energy among the evaluated compounds, with a docking score of -10.0 kcal/mol, indicating a strong and stable interaction with α-amylase (Greshock *et al.*, 2007). This compound forms a carbon-hydrogen bond with GLY306 and a conventional hydrogen bond with ASP300 at a distance of 1.93 Å through its amine and carbonyl groups, respectively. In addition, Mol7 establishes several hydrophobic contacts of the alkyl and π-alkyl types involving multiple residues, which collectively contribute to the stabilization of the ligand within the catalytic pocket.

Mol22 and **Mol23** were identified as chlororesistoflavin A and chlororesistoflavin B, respectively. These polyketide metabolites share highly similar chemical structures, differing mainly in the position of the chlorine atom on the aromatic ring. Both compounds exhibited identical binding energies of -9.5 kcal/mol and displayed comparable binding modes within the enzyme active site. Their interactions are mainly dominated by hydrophobic contacts, together with a conventional hydrogen bond formed with the catalytic residue ASP197.

Mol2, corresponding to 1-hydroxy-1-norresistomycin, Mol19 (talaromanloid A), and Mol20 (macrosporusone D) showed closely related binding energies of -9.2, -9.4, and -9.0 kcal/mol, respectively. In the case of Mol2, the hydroxyl group establishes a conventional hydrogen bond with GLN63, accompanied by hydrophobic interactions with HIS305, TRP58, and TRP59.

Mol19 interacts predominantly through hydrophobic π-alkyl and alkyl contacts, along with hydrogen bonds involving GLU233, TYR151, and ASP197. However, the presence of an unfavorable interaction may slightly reduce its overall binding stability.

Mol20 binds within the catalytic pocket mainly through hydrophobic interactions similar to those observed for Mol19, involving LEU162, LEU165, ALA198, and HIS305, and additionally forms a conventional hydrogen bond with HIS305.

Mol18 and **Mol21**, both belonging to the polyketide class, exhibited moderate and comparable binding energies of -8.5 kcal/mol. Despite their similar affinities, they displayed distinct interaction patterns within the catalytic pocket. Mol18 forms two conventional hydrogen bonds with ARG195 and ASP300, together with four hydrophobic interactions of the π-π stacked and alkyl types. In contrast, Mol21 establishes one conventional hydrogen bond and a π-anion interaction with ASP300, along with three hydrophobic interactions including π-sigma, alkyl, and π-alkyl contacts. An additional unfavorable interaction with GLU233 was observed, which may slightly decrease the binding efficiency. Nevertheless, the ligand remains well positioned within the catalytic region of the enzyme.

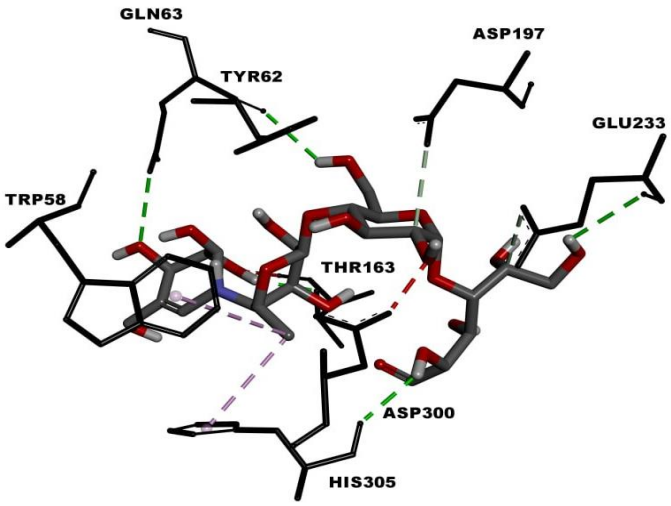
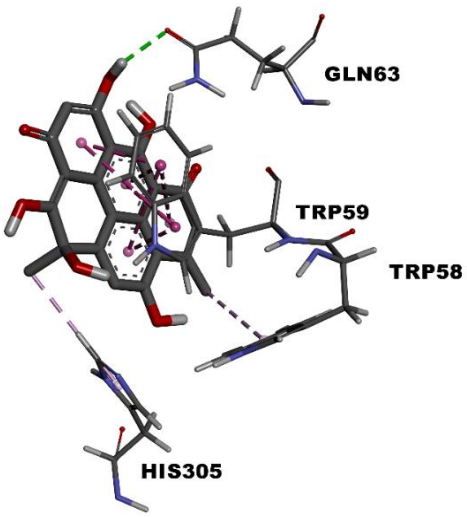
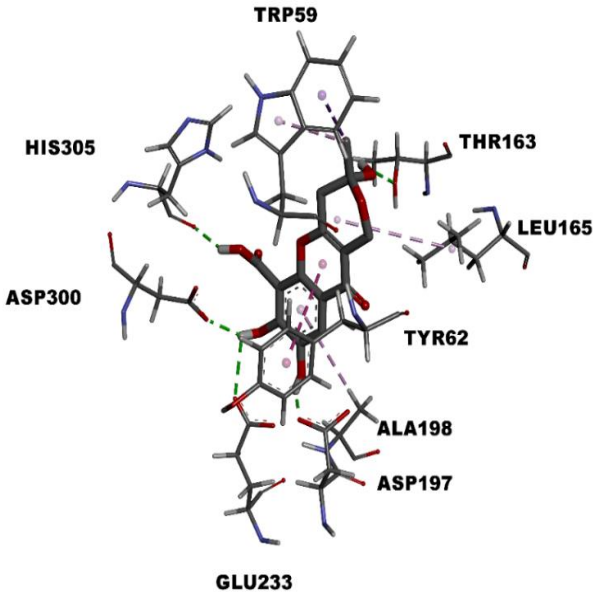
Mol6, corresponding to penialidin A, is a polyketide characterized by a central aromatic ring. This compound demonstrated a strong binding profile by forming five conventional hydrogen bonds involving key catalytic residues ASP197, GLU233, and ASP300, as well as HIS305 and THR163. Additionally, it establishes several hydrophobic interactions including π-sigma, π-π T-shaped, alkyl, and π-alkyl contacts. These extensive interactions contribute significantly to the stabilization of the complex.

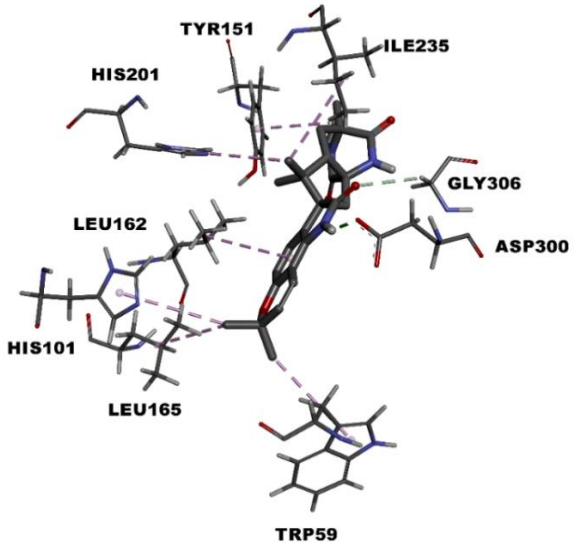
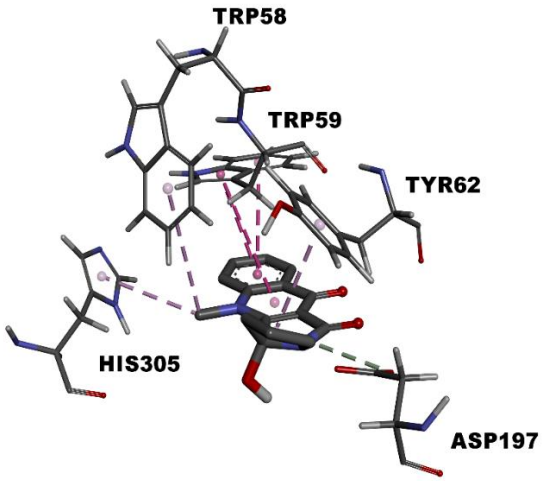
Mol15 and **Mol17** correspond to austalide P and penicillol A, belonging to the terpenoid and polyketide classes, respectively. Both compounds exhibited similar binding energies of -8.1 kcal/mol. Mol15 was the only compound among those evaluated that did not form conventional hydrogen bonds with residues of the catalytic site. Instead, its stabilization relied mainly on hydrophobic interactions and a π-anion interaction with ASP300. In contrast, Mol17 established multiple hydrogen bonds and hydrophobic contacts involving key residues such as TRP59, GLN63, LEU162, LEU165, and ASP300, contributing to its binding stability.

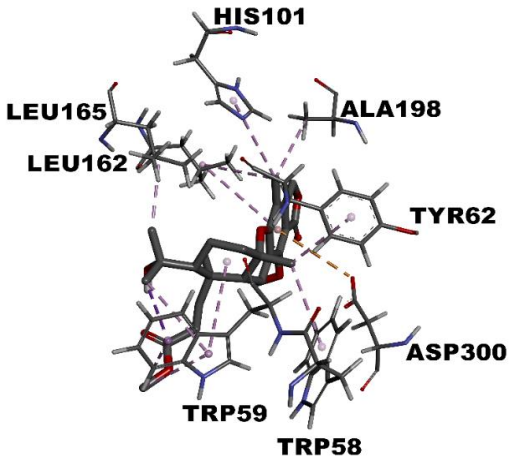
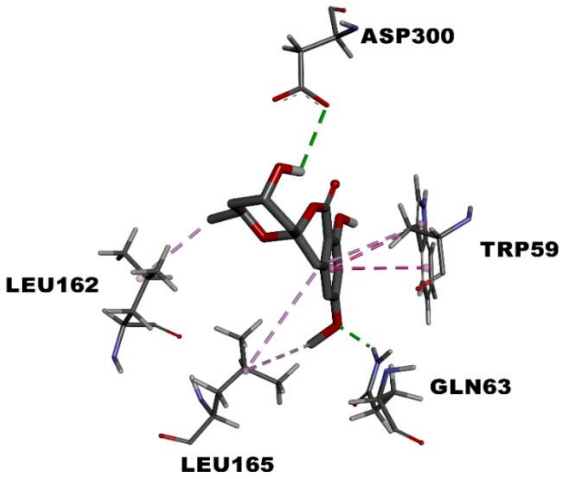
Finally, **Mol14** displayed the lowest affinity among the evaluated compounds targeting human pancreatic α-amylase, with a binding energy of -8.0 kcal/mol. Despite this relatively higher docking score, which is better than the control, it forms a carbon-hydrogen bond with

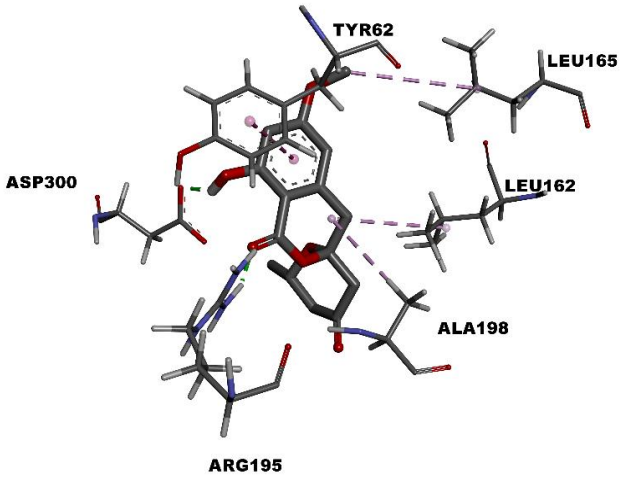
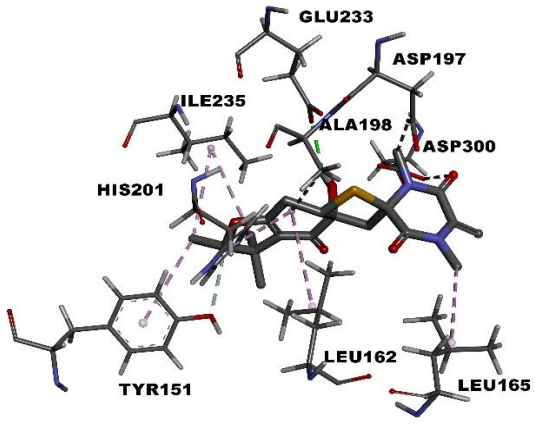
ASP197 and several hydrophobic interactions, including π-π stacking with TRP59 and π-alkyl interactions with TRP58, HIS305, and TYR62. However, the absence of conventional hydrogen bonds may explain its comparatively weaker binding affinity.

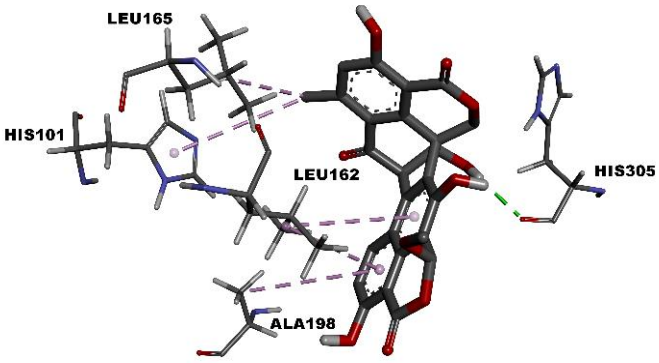
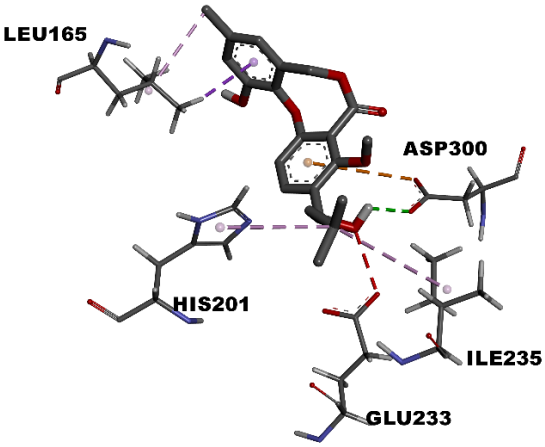
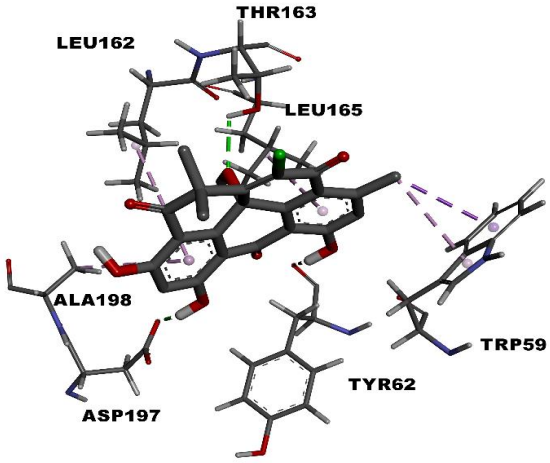
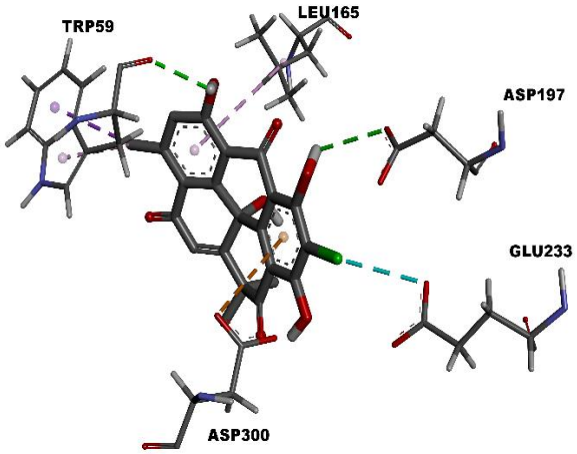
Table 4: The 3D structures of the best docking poses for the twelve compounds are represented in stick form. Conventional hydrogen bonds are highlighted in green, carbon-hydrogen bonds in gray, while hydrophobic and electrostatic interactions are depicted in purple and orange, respectively.

Control	
	
Mol 2	**Mol 6**
	
1-hydroxy-1-norresistomycin	**Penialidin A**

Mol 7	Mol 14
	
Notoamide B	Oxypenicinoline A

Mol 15	Mol 17
	
Austalide P	Penicillol A

Mol 18	Mol 19
	
Penicillol B	Talaromanloid A

Mol 20	Mol 21
	
Macrosporusone D	**Penicillide**
Mol22	**Mol23**
	
Chlororesistoflavins A	**Chlororesistoflavin B**

Overall, the majority of the studied compounds exhibit favorable pharmacokinetic and safety profiles, particularly in terms of intestinal absorption, metabolic stability, and low predicted toxicity. However, differences in membrane permeability, tissue distribution, and cytochrome P450 inhibition suggest that some compounds may require further optimization or experimental validation to fully assess their pharmacokinetic behavior. Additionally, molecular docking analysis revealed that the twelve candidates demonstrated potential inhibitory effects against HPA, as they showed the lowest binding energies compared to reference drug Acarbose, which

suggesting a stable binding mode and potential ability to inhibit the targeted enzyme, thereby the selected compounds could reduce carbohydrates digestion and help control postprandial blood glucose levels in diabetic patients.

CONCLUSION AND PERSPECTIVES

Type 2 diabetes mellitus is a complex and widespread metabolic disorder that affects millions of people worldwide. Although several oral and injectable medications are currently available to control blood glucose levels and reduce diabetes-related complications, many of these treatments are associated with undesirable side effects. Therefore, the discovery of safer and more effective therapeutic alternatives remains an important research priority.

The marine environment represents an exceptionally rich reservoir of biodiversity, providing numerous organisms capable of producing structurally diverse secondary metabolites with significant biological activities. These marine-derived compounds have attracted considerable interest as potential sources of novel therapeutic agents for various diseases, including diabetes.

In the present study, we aimed to identify potential antidiabetic compounds derived from marine organisms by predicting their inhibitory activity against human pancreatic α-amylase (HPA), a key enzyme involved in carbohydrate digestion. Three marine fungal species and one bacterial species were selected as sources of bioactive metabolites. A systematic stepwise strategy was employed, including literature-based compound selection, toxicity and pharmacokinetic evaluation, and MD analysis to investigate ligand-enzyme interactions.

Initially, 69 secondary metabolites previously isolated, purified, and chemically characterized from marine organisms were collected from the literature. These compounds were subjected to toxicity prediction, allowing the exclusion of molecules with carcinogenic potential or high risk of hERG channel inhibition. As a result, 23 molecules with acceptable safety profiles were retained and further evaluated for their pharmacokinetic properties using the PreADMET web server. MD analysis subsequently identified 12 compounds exhibiting stronger binding affinities toward HPA compared with the reference inhibitor acarbose. The pharmacokinetic assessment indicated that most of the selected marine metabolites possess favorable ADMT characteristics, particularly with respect to intestinal absorption, blood-brain barrier permeability, and metabolic interactions. Among them, Mol7, Mol22, and Mol23

demonstrated the strongest inhibitory potential against HPA, with binding energies of -10.0, -9.5, and -9.5 kcal/mol, respectively. Moreover, all twelve selected metabolites displayed stronger binding affinities than the control ligand acarbose, supported by the formation of key stabilizing interactions within the catalytic pocket of the enzyme.

Overall, the combination of favorable pharmacokinetic properties, acceptable toxicity profiles, and strong docking performance suggests that marine-derived secondary metabolites may represent promising candidates for the development of novel inhibitors targeting human pancreatic α-amylase for the management of T2DM.

As a future perspective, molecular dynamics simulations (MDS) could be conducted to further evaluate the stability and dynamic behavior of the most promising complexes under physiological conditions. In addition, further experimental validation through *in vitro* and *in vivo* studies will be essential to confirm the therapeutic potential of these compounds.

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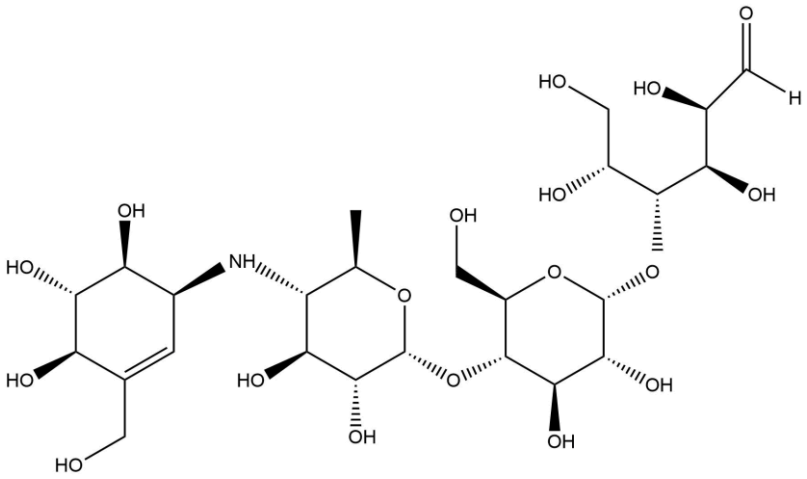
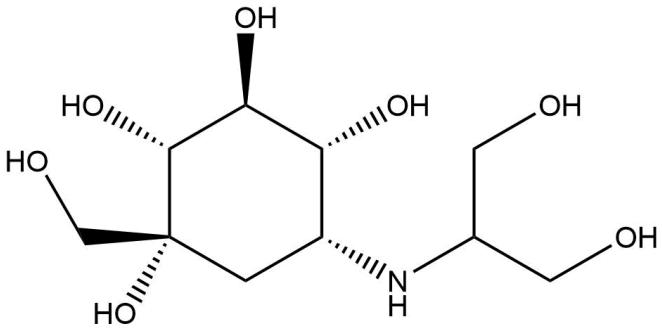
APPENDICES

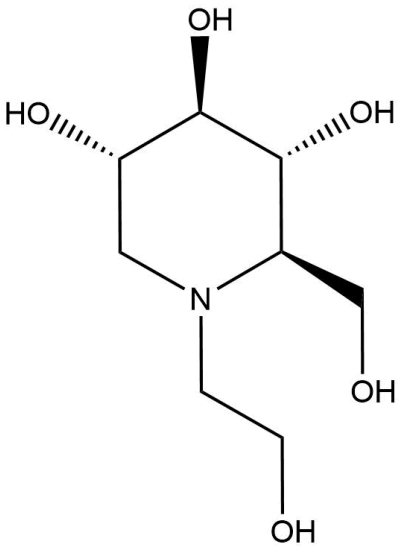
Appendix 1: 2D structure of Acarbose, Voglibose, and Miglitol.



Acarbose



Voglibose

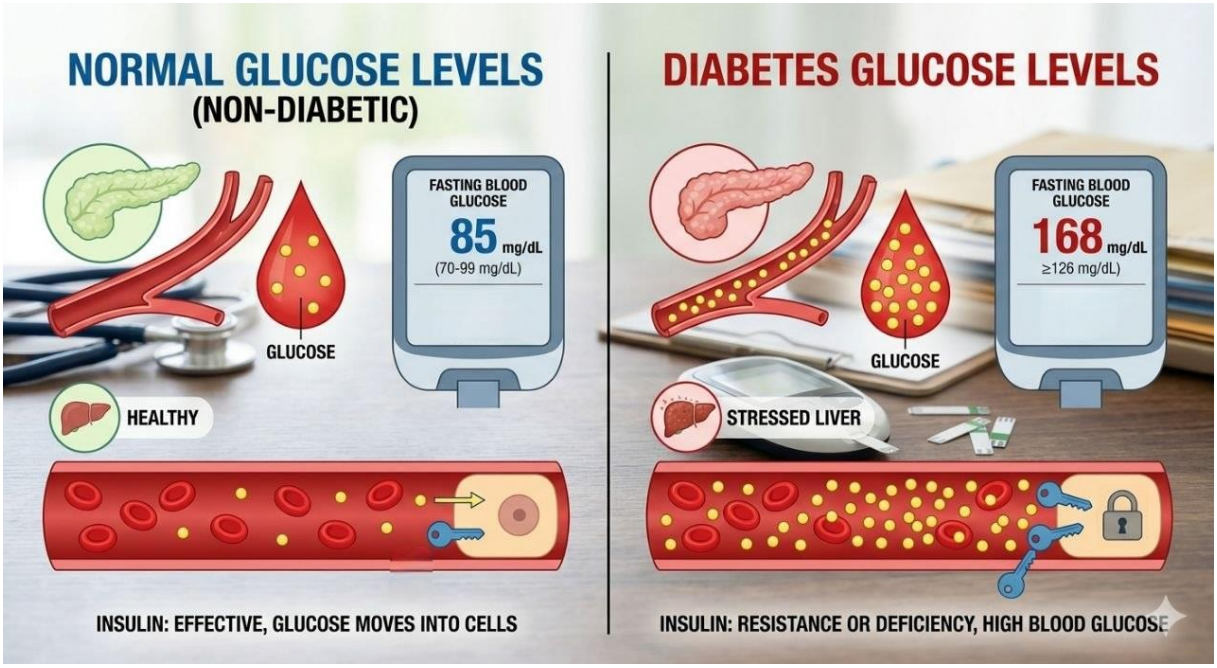


Miglitol

Appendix 2: Structural information on HPA available in the PDB.

PDB Code	Ligands	Resolution	Organisms	Mutation	References
3BAJ	ARE, NAG, NO3, Ca^{2+}	2.10 Å	Homo sapiens	NO	**(Maurus *et al.*, 2008)**
1B2Y	Ca^{2+}, Cl^-	3.20 Å	Homo sapiens	NO	**(Nahoum *et al.*, 2000)**
5VA9	Ca^{2+}, Cl^-	2.55 Å	Homo sapiens	NO	**(Goldbach *et al.*, 2019)**
2QMK	NAG, NO3, Ca^{2+}.	2.30 Å	Homo sapiens	NO	**(Maurus *et al.*, 2008)**

Appendix 3: Difference of the blood glucose level in normal and diabetic person



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Département de Biologie

جامعة غرداية

Université de Ghardaïa

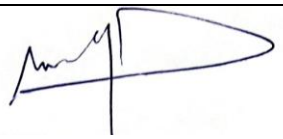
كلية علوم الطبيعة والحياة وعلوم الأرض

قسم البيولوجيا

Ghardaïa le :22/06/2026

Rapport : Correction du mémoire

Enseignants Chargé de la correction : Mr : LINANI Abderahmane

Nom et prénom l'examinateur 1	Nom et prénom de l'examinateur 2	Nom et prénom de président
Zineddine BENBEKHTI	/	**Mahfoud BAKLI**
Signature de	Signature	Signature
	/	

Thème :

In silico study of the anti-amylase activity of certain metabolites derived from marine organisms

Après les corrections apportées au mémoire, les étudiantes :

Yousra TFYECHE et **Sana TIRICHINE**

Sont autorisées à déposer le manuscrit au niveau du département.

Signature