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## ABSTRACT

The worldwide diabetes pandemic of **Type 2 diabetes mellitus (T2DM)** has become **the most common metabolic disease** which affects global populations as **the number of affected individuals is expected to reach 700 million people by 2045**. **Dipeptidyl peptidase-4 (DPP-4) inhibitors**, or gliptins, **have emerged as** an important pharmacological class for T2DM management by extending the bioavailability of **endogenous glucagon-like peptide-1 (GLP-1)**. The three gliptins that exist on the market include sitagliptin and saxagliptin and linagliptin but these medications cause harmful effects and create financial difficulties for patients **in low- and middle-income countries**. This situation creates **a need to study** natural products because they might provide better safety and cheaper development expenses.

Marine phlorotannins function as a unique type of polyphenolic compound which brown algae from the class Phaeophyceae use to create their own biosynthetic pathways. Marine phlorotannins show multiple biological activities which include their ability to fight diabetes and reduce inflammation and their antioxidant properties. The research team has not yet conducted a complete investigation into marine phlorotannins as potential lead substances for drug development.

The thesis establishes an entire in silico drug discovery process assessment for five marine phlorotannins which include phloroglucinol, eckol, dioxinodehydroeckol, fucodiphloroethol G, and phlorofucofuroeckol A as they interact with DPP-4 found in humans. Through AutoDock Vina molecular docking which operates with PyRx software phlorofucofuroeckol A showed the strongest binding capacity because it achieved **a binding energy of -10.2 kcal/mol** which exceeded **the binding energy of the FDA-approved drug sitagliptin** which reached **-9.2 kcal/mol**. The protein-ligand interaction analysis showed that BIOVIA Discovery Studio confirmed permanent binding with the DPP-4 active site through Glu205 Glu206 Tyr662 Tyr666 Trp659 catalytic residues.

The researchers conducted extensive computational ADMET testing through SwissADME and pkCSM to evaluate all candidate phlorotannins. The drug-like properties of Phlorofucofuroeckol A meet both Lipinski and Veber standards and it shows predicted oral bioavailability which is considered acceptable together with a safe toxicity profile. The comparative assessment between this marine natural product and sitagliptin shows its potential for practical application. The combined research results establish phlorofucofuroeckol A as a strong candidate for molecular dynamics simulations and in vitro enzymatic validation studies.

## CHAPTER 1: INTRODUCTION

### 1.1 THE GLOBAL BURDEN OF TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder which develops continuously and causes permanent high blood sugar because of insulin secretion problems and insulin sensitivity issues and their combined effects. More than 90% of the people worldwide who suffer from diabetes are affected by this condition and it has emerged as a major public health threat for the 21st century. According to the International Diabetes Federation (IDF), there are 537 million adults with diabetes in the world in 2021 and this figure will rise to 783 million by 2045, predominantly in low and middle-income countries [1]. Diabetes has a significant impact on health care facilities globally with various macro and microvascular Diabetes complications such as coronary artery disease, diabetic nephropathy, diabetic peripheral neuropathy and diabetic retinopathy. [2].

The financial burden of treating diabetes is staggering as well. Medical financial expenditures attributable to diabetes directly cost the world at about USD 966 billion in 2021. The high and frequent expenses of routinely treating patients with pharmacotherapy, frequent patient hospitalizations, and chronic clinical care for secondary complications are major drivers of this massive economic drain.. Diabetes creates socioeconomic effects that extend beyond its direct costs because it reduces workforce productivity and increases employee absenteeism while it decreases people's capacity to live their lives [1].

There are several different classes of drugs in the typical armamentarium used to treat T2DM. Metformin is usually the initial treatment, and sometimes combined with sulfonylureas or SGLT-2 inhibitors. Some advanced glycemic control methods include GLP-1 receptor agonists in combination with dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 inhibitors which doctors refer to as gliptins have become a major focus in clinical research because their glucose-dependent action mechanism keeps patients at stable weight while their risk of inducing hypoglycaemia remains lower than that of sulfonylureas [3]. The primary issues about this treatment involve its cardiovascular safety and its pancreatitis risk and its cost issues which especially affect patients from developing countries [4].

The current situation creates a strong need to discover new DPP-4 inhibitors through natural product research because synthetic gliptins have safety issues and high production costs. The research evaluates marine phlorotannins as potential lead candidates in a computational drug discovery pipeline because they show binding affinity and can progress through the complete drug development process.

## 1.2 DPP-4 AS A VALIDATED PHARMACOLOGICAL TARGET

Dipeptidyl peptidase-4 (DPP-4; EC 3.4.14.5) which scientists refer to as CD26 functions as a serine protease which exists throughout the body to quickly break down incretin hormones. Normal systemic functions of active GLP-1 involve the induction of insulin secretion from pancreatic  $\beta$ -cells paired with the concurrent suppression of  $\alpha$ -cell glucagon release. Furthermore, this incretin hormone modulates postprandial homeostasis by slowing downward gastric clearance speeds and signaling satiety to the central nervous system. [5]. DPP-4 cleaves the two N-terminal amino acids from active GLP-1 (7–36 amide) within minutes of secretion, reducing its half-life to under two minutes and thereby limiting its insulinotropic effect. The validation of DPP-4 as a drug target is exceptionally well-supported. Genetic knockout mouse models deficient in DPP-4 exhibit improved glucose tolerance, enhanced GLP-1 levels, and protection against diet-induced obesity [6]. The first FDA-approved gliptin (2006) sitagliptin which acts as a DPP-4 inhibitor showed substantial HbA1c reduction during clinical trials which proved the target's translational validity. The human DPP-4 protein (UniProt P27487) operates as a type II transmembrane glycoprotein which exists in a homodimeric form. Each monomer contains an N-terminal transmembrane domain, a stalk region, and a large extracellular catalytic domain which contains the classic serine protease catalytic triad (Ser630, Asp708, His740). The active site contains a substrate recognition region which consists of two subsites, S1 and S2, that serve as the main interaction points for both natural substrates and artificial inhibitors [7].

The well-characterised three-dimensional architecture of DPP-4, with its multiple available high-resolution crystal structures in the Protein Data Bank (PDB), serves as an ideal target for structure-based virtual screening because this method functions as a fundamental component of contemporary computer-based drug discovery processes.

## 1.3 LIMITATIONS OF SYNTHETIC GLIPTINS: A CLINICAL AND TRANSLATIONAL PERSPECTIVE

The FDA and EMA have approved five DPP-4 inhibitors which include sitagliptin (Januvia®), saxagliptin (Onglyza®), linagliptin (Tradjenta®), alogliptin (Nesina®), and trelagliptin. The agents show effective treatment results with a safety profile that remains better than traditional oral hypoglycaemics for initial treatment periods, but their extended clinical application has exposed several major issues which need better treatment options [4].

The safety assessment process has identified post-marketing surveillance results which show that gliptin use raises both pancreatitis and pancreatic cancer risks although the direct link between these two conditions remains under investigation [8]. The SAVOR-TIMI 53 trial showed that saxagliptin treatment resulted in a higher rate of hospital admissions due to heart failure when compared to the placebo group, which led to regulatory label changes and ongoing cardiovascular outcome monitoring for all drugs in that particular category [9]. The study found that patients experienced hypersensitivity reactions together with nasopharyngitis and upper respiratory tract infections and urinary tract infections at rates that exceeded those observed in the placebo group.

The high cost of branded gliptins exists as an accessibility barrier which creates financial difficulties for patients throughout the world. The United States market shows that Sitagliptin has a wholesale acquisition cost which exceeds USD 400 per month and despite the launch of generic products after the patent expired their prices create difficulties for various healthcare systems. The synthetic agents classified as small-molecule gliptins which do not originate from natural sources face challenges because patients and prescribers prefer herbal and natural treatments in cultural contexts.

The existing restrictions demonstrate the requirement for DPP-4 inhibitors which originate from natural product scaffolds to create new drug development pathways that produce substances with fresh structural designs and improved safety standards and reduced manufacturing expenses and compatibility with traditional natural product medicinal systems. Marine phlorotannins represent exactly such a candidate class.

## 1.4 NATURAL PRODUCTS IN THE DRUG DISCOVERY PIPELINE

Natural products have always been the most successful source for finding new drugs through pharmacological research and this trend continues to persist today. In all the new chemical entities approved between 1981 and 2019, an analysis conducted by Newman and Cragg (2020) revealed that more than half of the approved drugs were either natural products, natural product derivatives or synthetic compounds designed on natural product scaffolds [10]. The amazing history includes the discovery of a number of 'signature' drugs, including those containing the penicillin, paclitaxel, morphine, artemisinin, and metformin, all of which stem from guanidine structures found in *Galega officinalis*.

The modern drug discovery pipeline for natural products typically encompasses several sequential stages: (1) compound identification and sourcing; (2) preliminary biological activity screening; (3) computational (in silico) evaluation including molecular docking and ADMET prediction; (4) in vitro biochemical validation including enzymatic assays; (5) cell-based efficacy and cytotoxicity studies; (6) in vivo pharmacokinetic and pharmacodynamic studies; and (7) clinical trials. The research process requires computational tools at stages 2 and 3 because they allow researchers to perform virtual screening tests which examine vast compound libraries against known targets before they conduct expensive laboratory tests [11].

The lead candidate functions as a vital element in pipeline-stage drug discovery because this compound needs to show target binding while meeting essential pharmacokinetic and drug-likeness standards that predict its ability to perform in live tests. Researchers use SwissADME and pkCSM to create computational tools which enable them to eliminate unpromising compounds during initial testing despite their strong in vitro binding results. The thesis applies this framework to the study of marine phlorotannins.

## 1.5 MARINE PHLOROTANNINS: SOURCES, CHEMISTRY, AND BIOLOGICAL ACTIVITY

The structurally unique class of marine polyphenolic compounds, called phlorotannins, are only produced in brown algae (Class Phaeophyceae) by the polyketide pathway. Phlorotannins are oligomeric and polymeric compounds composed of phloroglucinol (1,3,5-trihydroxybenzene) monomeric units, linked via aryl-aryl, ether or mixed linkages, whereas terrestrial tannins are derived from gallic acid (hydrolysable tannins) or flavan-3-ols units (condensed tannins) [12]. The biosynthetic origin gives phlorotannins structural properties that are characteristic of no other class of polyphenol from land plants, and are particularly unusual in terms of aromaticity and hydroxylation density.

The five phlorotannins examined in this study vary widely in their structural complexity, and include the major subclasses of phlorotannins found in brown algae: the monomeric phloroglucinol, the trimeric dibenzo-1,4-dioxin-type compound, eckol, the oxidation product dioxinodehydroeckol, and the fucodiphloroethol-class compound, fucodiphloroethol G, and the compound phlorofucofuroeckol A, which is a pentameric compound of the phlorofucofuroeckol-class.

Phlorotannins of *E. cava* are the most widely studied ones in terms of their biological properties. Potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibiting activity was demonstrated by several *Ecklonia* phlorotannins, comparable to the reference drug, acarbose, by Wijesekara et al., 2010 [13]. Inhibition of DPP-4 activity by phlorotannins has been identified in enzymatic assays systems, and phlorofucofuroeckol A has consistently been the most potent congener in several enzymatic and biological activity assays.

Although promising experimental results have resulted, a systematic screening and evaluation of marine phlorotannins in the context of the drug discovery pipeline (binding affinity, pharmacokinetics, drug-likelihood and comparative screening) has not been performed. The present thesis is an attempt to bridge that gap.

## 1.6 RATIONALE AND OBJECTIVES OF THE STUDY

The overarching and central translational question for the present thesis is whether marine phlorotannins meet the multiparametric criteria necessary to become lead candidate in a drug discovery pipeline aimed at developing inhibitors for the DPP-4 enzyme in T2DM. Although the molecular docking affinity is one important criteria, based on its own, a compound cannot be regarded as a viable lead; it should also have good oral bioavailability and house metabolic stability, tolerable toxicity, and follow drug-likeness rules.

The following are the objectives that the study will be followed to answer this question:

1. Using AutoDock Vina, perform molecular docking of five structurally diverse marine phlorotannins with a human DPP-4 crystal structure, predicting them for their predicted binding affinity.
2. Using BIOVIA Discovery Studio to characterise among each docked compound, its molecular interaction profile with catalytic and the substrate-binding residues of DPP-4.
3. To do full ADMET Profiling of all candidate phlorotannins for SwissADME and pkCSM to understand the pharmacokinetic viability and potential safety of each candidate.
4. To compare the most active phlorotannin to the FDA-approved gliptin sitagliptin in terms of both binding affinity and drug-likeness.
5. To situate the topperforming candidate(s) in a drug discovery pipeline framework and set up a path forward for further experimental validation.

## CHAPTER 2: REVIEW OF LITERATURE

### 2.1 DPP-4 ENZYME: STRUCTURAL AND FUNCTIONAL OVERVIEW

Thus, the present thesis would be centered around a key translational question – “Is the marine derived phlorotannins fit with the multiparametric criteria that needs to be met as a lead candidate in a drug discovery pipeline for the target of DPP-4 in T2DM?” Although molecular docking affinity is important, it isn't enough; the compound should also have a good oral bioavailability, metabolic stability, and low toxicity, and should meet the criteria for drug-likeness. **Dipeptidyl peptidase-4 (DPP-4; CD26) is composed of the** prolyl oligopeptidase family (S9B subfamily) characterized by the presence of a post-proline specificity where dipeptides are removed preferentially **from the N-terminus of** substrates when **the second amino acid** is proline or alanine [5]. This specificity is reflected in its primary endocrine substrates, GLP-1 and GIP, which both have a penultimate alanine.

Human **DPP-4 is a 766 amino acid** type II **transmembrane** glycoprotein which occurs as a membrane-bound glycoprotein in the kidney, liver, small intestine and immune cells, or as a soluble component detectable in plasma. It has a canonical  $\alpha/\beta$ -hydrolase-like catalytic domain with the **catalytic triad (Ser630–Asp708–His740) as well as** a propeller domain that acts as a substrate binding vestibule [7]. Two subsites, S1 (hydrophobic pocket lined by Phe357, Val207 and Tyr547 — the penultimate residue from the substrate fits here) and S2 (open, electropositive pocket between Glu205, Glu206, Tyr662, Tyr666 and Trp659 — is the principal position of synthetic inhibitors).

Residues Glu205 and Glu206 in the S2 subsite are more important, forming a "glutamate dyad" that provides a negatively charged area that is well ordered for binding with the positive charge on the pharmacophore of most clinically used DPP-4 inhibitors. Tyr662 and Tyr 666 provide aromatic stacking and hydrogen bonding capability, Trp659, provides  $\pi$ - $\pi$  and hydrophobic interactions with the aromatic scaffold of the inhibitor. **The active site of DPP-4 is extremely druggable with** the active site being well defined with an enclosed hydrophobic active site, as well as clear pharmacophoric requirements for productive binding [7].

### 2.2 CLINICALLY APPROVED DPP-4 INHIBITORS: MECHANISMS AND ADVERSE EFFECTS

Sitagliptin, saxagliptin, linagliptin, alogliptin and trelagliptin are 5 DPP-4 inhibitors available and in clinical practice. Although they have the same target and overall mechanism of action, they have vastly different chemical scaffolds, protein binding characteristics, metabolic pathways, and adverse effect profiles [4]. Sitagliptin (Januvia®, Merck), the first drug approved by FDA for DPP-4 (2006), is a highly selective fluorine-containing triazolopyrazine inhibitor of DPP-4, which shows **high selectivity for DPP-4 over related enzymes DPP-8 and DPP-9**. It is excreted renally and needs dosage adjustment **in patients with chronic kidney disease** [3]. Saxagliptin (Onglyza®, AstraZeneca/BMS) is an hydroxyadamantane modified pyrrolidine which **forms a covalent, reversible interaction with Ser630, the catalytic Serine residue**. **In the SAVOR-TIMI 53 trial, saxagliptin was found to be associated with an increased risk of hospitalisation for heart failure and subsequently an FDA black box warning was placed on the drug** [9].

Linagliptin (Tradjenta®, Boehringer Ingelheim/Eli Lilly) has an unusual pharmacokinetics profile, being excreted primarily in the bile and faeces, thus avoiding the need to adjust the dose in patients with all stages of renal impairment [3]. It contains xanthine structure which is able to interact with DPP-4 by ionic, hydrogen bond and hydrophobic interactions. Although gliptins have several mechanistic-based advantages, there are several concerns across the class which include risks of pancreatic inflammation (pancreatitis), joint pain (arthralgia), and there is emerging post-marketing data on bullous pemphigoid, a rare, but severe dermatological adverse effect that is nearly unknown [8].

Although the cost of gliptin therapy is limited, it is still an access problem in developing countries, where the prevalence of T2DM is increasing most rapidly in pharmaco-economic terms. This has led to a greater interest in natural product-derived leads which may be used for the development of cheap drugs or as actual therapeutic agents within an integrative medicine context.

### 2.3 PHLOROTANNINS AS ANTIDIABETIC AGENTS: EXPERIMENTAL EVIDENCE

There has been significant experimental evidence for the anti diabetic effect of phlorotannin in the last two decades. Several phlorotannins from *Ecklonia cava*, such as dieckol, phlorofucofuroeckol A and eckol, were shown to be highly potent inhibitors of  $\alpha$ -glucosidase and  $\alpha$ -amylase in vitro, with IC50 values comparable to that of the clinical reference drug acarbose [13]. The discovery of these carbohydrate-hydrolysing enzyme inhibitory activities is complementary inhibitors for DPP-4 to regulate post-prandial glycaemia.

To specifically discuss DPP-4, Lopes et al. (2019) performed a systematic review of the bioactivities of phlorotannins relevant to the hypoglycaemic control; among them, DPP-4 inhibitors were highlighted. Compared to the enzymatic assays, the authors concluded that the phlorotannins of the Fucales order displayed particularly potent DPP-4 inhibitory activity with phlorofucofuroeckol A being the consistent most potent congener [14]. Most of the experimental studies have reported IC50 data obtained from the cell-free enzymatic assays, however, and the translatability of these bioactivity data has not yet been determined.

A critical translational gap therefore exists, as the literature on the biological activity of phlorotannins shows that they were able to inhibit the activity of DPP-4 in vitro, but no systematic analysis revealed the pharmacokinetic and drug-likeness properties that are required for in vivo efficacy. This thesis fulfills that need by implementing a drug discovery pipeline approach, which combines drug docking information with extensive ADMET profiles.

### 2.4 COMPUTATIONAL DRUG DISCOVERY: PIPELINE AND VALIDATION STANDARDS

The use of computational drug discovery in the early discovery stage has revolutionized the drug development pipeline due to the ability to rapidly and cost effectively screen large compounds libraries against knowledge controlled targets before reaching experimental testing. It includes structure-based virtual screening, pharmacophore modelling, quantitative structure-activity relationship (QSAR) modelling, and more recently, the machine learning-based approaches [11].

Molecular docking is a computational method used in this study that provides the most favorable orientation (pose) of a small molecule bound to a protein to the active site and also estimates the

54 free energy of binding of the small molecule binding the protein. The docking algorithm used in this study through PyRx is called AutoDock Vina and optimises the conformation of the ligand over the specified grid box using a gradient optimisation algorithm, providing a number of poses based on the estimated binding free energy (eBFE) [15]. The binding free energy output (kcal/mol) is a quantitative indicator of binding affinity of a peptide, where a more negative value suggests a more strongly predicted binding.

The protocols are usually validated by repeated docking of the native co-crystallised ligand and/or a known inhibitor which would recapitulate the crystallographic structure (RMSD < 2.0 Å). The practice for virtual screening is to use a reference drug as a guideline drug and the docking score of the guanfacine (-9.2 kcal/mol) is used as the threshold to screen the candidate compounds as phlorotannins.

## 2.5 ADMET PROFILING IN EARLY DRUG DISCOVERY

The drug discovery process uses ADMET profiling as an essential tool for evaluating lead candidates. The research shows that most drugs fail during clinical development because they have poor pharmacokinetic properties or toxic effects rather than their inability to bind to targets which creates the historical observation that 'most drugs fail in the clinic not because they don't bind but because they don't behave' [16].

30 The Rule of Five establishes the fundamental standards for evaluating oral drug-likeness through four criteria which require molecular weight to be less than or equal to 500 Da and LogP to stay below 5 and hydrogen bond donors to remain under 5 and hydrogen bond acceptors to stay below 10 [17]. Veber's rules establish rotatable bond limits at 10 and total polar surface area (TPSA) limits at 140 Å<sup>2</sup> to create new oral bioavailability prediction methods. The compounds that break these rules will have their gastrointestinal absorption and membrane permeability capabilities predicted to experience challenges.

SwissADME, is a web application for rapid ADMET prediction that evaluates Ro5 compliance, gastrointestinal absorption, blood-brain barrier permeability, substrate probability for the P-glycoprotein transporter, and cytochrome P450 enzyme inhibition profiles [18]. In addition, pkCSM applies a graph-based signature approach for predicting toxicity parameters such as AMES mutagenicity, hERG cardiac toxicity, hepatotoxicity, and LD50 [19]. These two approaches enable an early evaluation of ADMET properties, providing valuable information on whether to proceed or not in computational drug discovery studies.

## CHAPTER 3: MATERIALS AND METHODS

### 3.1 PROTEIN STRUCTURE RETRIEVAL & PREPARATION

The crystal structure of human dipeptidyl peptidase-4 (DPP-4) PDB ID: 1X70 was downloaded from the Protein Data Bank ( <https://www.rcsb.org> ). A high crystallographic resolution of the structures and the presence of a well-defined cavity and catalytic residues fully resolved in the active site were chosen as selection criteria. It was checked for completeness by examining the REMARK section of the PDB file and the quality assessment of the electron density map found in the PDB validation report.

Protein preparation was done as per standard pre-docking procedure. To remove such computational artefacts, all crystallographic water molecules and non-structural ions were removed during the grid generation. The protein was assigned Kollman partial charges and polar hydrogens were added to correctly depict the electrostatic environment in the protein's active site. The preprocessed protein was saved in PDBQT format including atom types and partial charges used by the AutoDock Vina scoring function. The integrity of the active site cavity was verified by visual inspection in BIOVIA Discovery Studio before docking, especially the S1 and S2 subsites.

### 3.2 LIGAND SELECTION AND PREPARATION

Five marine phlorotannins were chosen as potential ligands, namely the monomeric precursor unit, phloroglucinol, the dibenzodioxin trimer, eckol, the oxidised variant, dioxinodehydroeckol, the difucodiphloroethol tetramer, fucodiphloroethol G and the complex pentameric phlorofucufuroeckol congener, phlorofucufuroeckol A. The selection was made to represent the entire structural diversity space of phlorotannins, from the simplest monomeric unit to the highest level of polymerisation of the aromatic systems.

All five phlorotannins were downloaded in SDF format from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) as three-dimensional structures. The same source was used for the same compound and used as a drug control throughout the study (Sitagliptin, PubChem CID: 4369359). Ligand structures were uploaded into PyRx and then geometry optimised using UFF (Universal Force Field) energy minimisation protocol to low energy conformations. A set of torsion trees were defined to find rotatable bonds and allow flexible ligand docking. All the ligands were converted to PDBQT for compatibility with AutoDock Vina.

### 3.3 MOLECULAR DOCKING PROTOCOL

Molecular docking was carried out with the AutoDock Vina algorithm embedded in the PyRx virtual screening platform (version 0.8). The docking grid box was positioned about the catalytic region of the active site of DPP-4 with centroid position at  $X = 15.9277 \text{ \AA}$ ,  $Y = 29.1036 \text{ \AA}$  and  $Z = 58.5709 \text{ \AA}$ . Box side lengths along the X, Y and Z dimensions were of 30.6782  $\text{\AA}$ , 30.8812  $\text{\AA}$  and 30.4579  $\text{\AA}$ , respectively, allowing complete coverage of both the S1 and S2 subsites, as well as extended conformations of the larger phlorotannin molecules. A search volume of 8 was used with the exhaustiveness parameter to sample the conformational space within the defined search volume in detail. A total of nine binding modes (poses) were obtained for each of the ligands, and

the pose with the negative most predicted binding free energy was chosen as the binding conformation representative of each ligand. The flexible part of the receptor (ligands without rotatable bonds) was held fixed, but all rotatable bonds in the ligand were allowed full torsional flexibility. The reference drug sitagliptin was docked under the same conditions and in the same manner such that it could be directly compared with the binding affinity of our drug.

### 3.4 BINDING AFFINITY EVALUATION

Binding affinity was quantified, and expressed as the predicted Gibbs free energy of binding ( $\Delta G$ , kcal/mol) using the AutoDock Vina empirical scoring function. The binding energy for the pose with the highest score was taken for each compound as the main affinity measure. Descriptive statistics (mean  $\pm$  standard deviation) of the nine docking poses per compound were used to evaluate docking pose reproducibility and conformational sampling robustness. Inter-pose standard deviations of the lower values signify that the compound binds to the protein in the same fashion no matter the conformation it started in and therefore provide further evidence for how promiscuous the binding prediction is.

The compounds were in ranked order based on top pose binding energy and were separated according to predicted relative value to the control compound ( $-9.2$  kcal/mol) sitagliptin. Phlorotannins, with binding energies equal to or more negative than sitagliptin, were flagged as high priority candidates for getting through ADMET analysis, and honestly like some kind of progression there.

### 3.5 ADMET PROFILING VIA SWISSADME AND PKCSM

ADMET data was produced in detail through the use of two different complimentary Web based platforms. Physicochemical properties such as the molecular weight (MW), hydrogen bond donor count (HBD), the hydrogen bond acceptor count (HBA) and the number of rotatable bonds were calculated using SwissADME (<https://www.swissadme.ch/>). The topological polar surface area (TPSA) was calculated using SwissSARPFITS (<https://www.swissadme.ch/SwissSARPFITS>). Lipinski's Rule of 5 (Ro5) [17] rule and Veber's rule and Ghose filter were used to test for drug-likeness. SwissADME was used to extract data such as gastrointestinal absorption, permeability of the blood-brain barrier, substrate properties of P-glycoprotein, and inhibition profile of different CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4).

To link the product to toxicity data from the most similar products, toxicity profiling was performed via pkCSM (<https://biosig.lab.uq.edu.au/pkcsm/>), which uses graph-based signatures trained on experimental pharmacokinetic and toxicity data [19]. The parameters measured involved AMES mutagenicity, maximum tolerated dose (human), inhibition of hERG I and hERG II (surrogate markers of cardiac QT prolongation), hepatotoxicity and skin sensitisation. To allow for direct cross-compound comparisons, all the analyses performed were done for all five phlorotannins and sitagliptin at once.

### 3.6 COMPARATIVE ANALYSIS WITH SITAGLIPTIN

A head-to-head comparative pharmacological profile was constructed for the top-performing phlorotannin(s) relative to sitagliptin. The comparison encompassed: (1) predicted binding affinity (kcal/mol); (2) interaction type and residue coverage at the DPP-4 active site; (3) Ro5 and Veber compliance; (4) predicted gastrointestinal absorption; (5) hepatotoxicity and hERG risk; and (6) CYP inhibition liabilities. This kind of comparison gives the basis for the pipeline stage lead candidate ranking and kind of points out where phlorotannins could maybe bring pharmacological benefits over the approved standard of care that is already in place.

## CHAPTER 4: RESULTS

### 4.1 MOLECULAR DOCKING RESULTS

All of the five phlorotannins and reference drug (sitagliptin) have been successfully docked to the active site of DPP-4. Table 1 shows the binding energies and statistical descriptors at the top position for each pose.

Compound	Best Binding Energy (kcal/mol)	Mean $\pm$ SD (9 Poses, kcal/mol)	Rank Sitagliptin vs.
Phloroglucinol	-5.3	-5.08 $\pm$ 0.19	Below control
Eckol	-8.5	-7.86 $\pm$ 0.43	Below control
Fucodiphloroethol G	-8.3	-8.01 $\pm$ 0.35	Below control
Dioxinodehydroeckol	-9.0	-8.66 $\pm$ 0.24	Comparable
Sitagliptin (Control)	-9.2	-8.64 $\pm$ 0.32	Reference
Phlorofucofuroeckol A	-10.2	-9.56 $\pm$ 0.33	Exceeds control

Table 1. Binding energies (kcal/mol) and mean  $\pm$  SD for the top nine docking poses for each compound.

The phlorofucofuroeckol A showed the lowest binding energy of -10.2 kcal/mol, 1.0 kcal/mol lower than the binding energy of the control (sitagliptin, -9.2 kcal/mol) according to the AutoDock Vina scoring function. Dioxinodehydroeckol and sitagliptin exhibited similar binding spectroscopic values with values of -9.0 and -9.2, respectively, while eckol and fucodiphloroethol G exhibited moderate binding values of -8.5 and -8.3, respectively. Phloroglucinol had the lowest binding energy (-5.3 kcal/mol) in accordance with the least molecular complexity which is the monomeric building block.

The standard deviations in 9 poses were all found to be quite low across the nine different binding poses (range of 0.19–0.43 kcal/mol), suggesting that each compound, under the current docking protocol, was consistently bound in DPP-4 in a particular binding pose. The lowest inter-poses variability was seen here for phloroglucinol (SD = 0.19) and dioxinodehydroeckol (SD = 0.24) indicating very stable binding modes for these compounds.

### 4.2 PROTEIN-LIGAND INTERACTION PROFILES

Interaction analysis with the active site residues of the enzyme DPP-4 in BIOVIA Discovery Studio provided a lead to compound-specific interactions with DPP-4 residues of the active site. Three types of non-covalent interactions were used to study interactions quality and diversity: hydrogen bonds, hydrophobic contacts and aromatic ( $\pi$ - $\pi$ ) stacking interactions.

#### 4.2.1 PHLOROGLUCINOL

Phloroglucinol made primary hydrogen bonding interactions with Glu205 and Glu206, only engaging in binding into the S2 subsite. The orientation from the 2D binding interaction map shows that there is no penetration into the S1 subsite and the interaction is shallow. The predicted binding affinity is significantly lower for this monomer (see Figure 1) because of the limited surface area of the monomer in comparison to the spatial size of the DPP-4 active site cavity.

#### 4.2.2 ECKOL

Eckol has an improved interaction profile compared to phloroglucinol, and hydrogen bonded to Glu205, Glu206, and Tyr662. With the extended dibenzodioxin ring scaffold, partial overlap with both S1 and S2 subsites was allowed, while in addition to these beneficial hydrophobic contacts, larger groups of atoms were able to penetrate the binding cleft. The binding energy improvement of about 3.2 kcal/mol with this type of linkage seems to be requisite for bridging the two subsites (Figure 2).

#### 4.2.3 PHLOROFUCOFUROECKOL A

The compound phlorofucofuroeckol A showed the widest interaction pattern of all the compounds studied. Several hydrogen bonds to Tyr662, Glu205 and Glu206 were found, and extensive aromatic stacking interactions with Tyr666 and Trp659 were identified. This is a compound that was successfully active at both the S1 and the S2 subsite, being that the cavity in the active site of DPP-4 is a pentameric structure that binds the compound optimally, while its structure bears a complementarity to the active site. The multivalency and breadth of these interactions directly contribute to its highest binding affinity in all of the studies (−10.2 kcal/mol) and most complete binding site engagement of all candidates (Figure 3).

#### 4.2.4 DIOXINODEHYDROECKOL

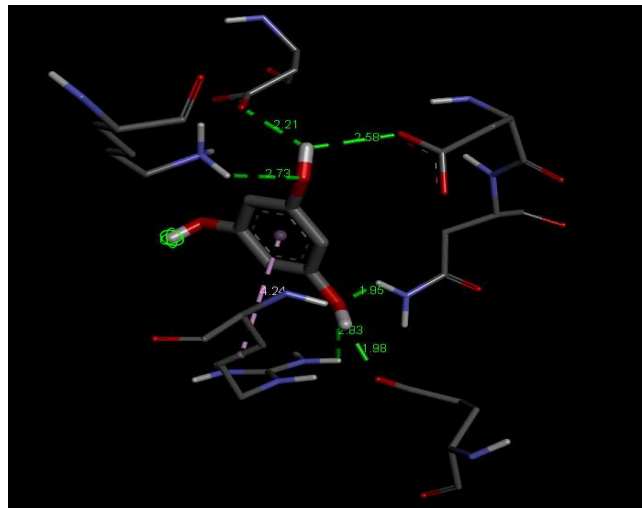
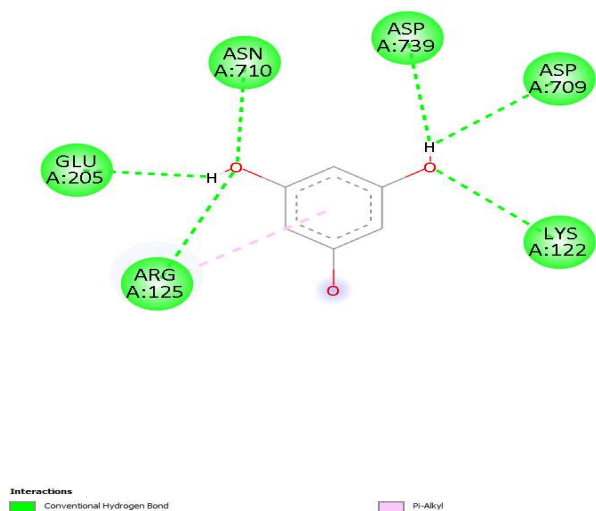
Among all of these compounds, dioxinodehydroeckol was found to have an even more stable binding mode that formed a hydrogen bond with Glu205, Glu206, and Tyr666, in addition to the  $\pi$ – $\pi$  stacking interaction with Trp659. The 3D binding conformation showed these to be the lowest SD (0.24) and binding energy (−9.0 kcal/mol. with only little unfilled volume of the pocket, close to that of sitagliptin (Figure 4).

#### 4.2.4 FUCODIPHLOROETHOL G

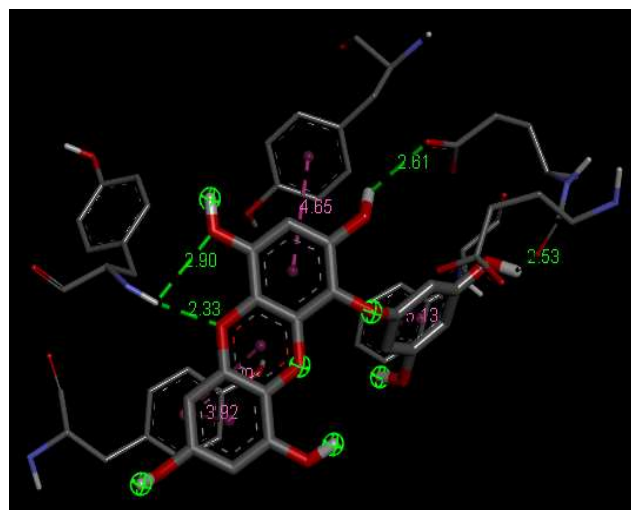
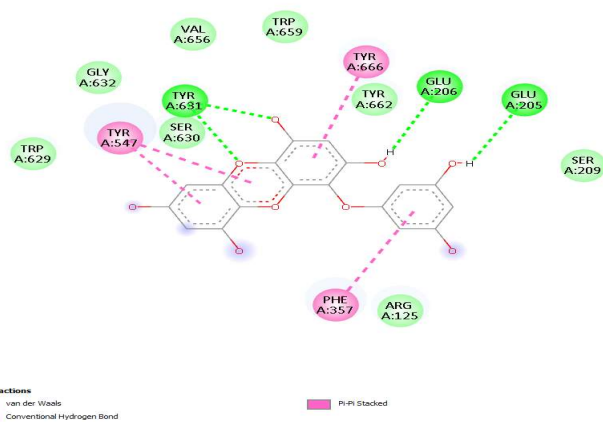
Fucodiphloroethol G formed hydrogen bonds with Glu205, Glu206, and Tyr547, alongside a more hydrophobic type of interaction. Even though its molecular size was sort of intermediate compared with phlorofucofuroeckol A, it still showed a weaker binding affinity of −8.3 kcal/mol. That may be because of less-than-ideal geometric match between its linear fucodiphloroethol skeleton and the three dimensional shape of the DPP-4 pocket, (Figure 5)

3

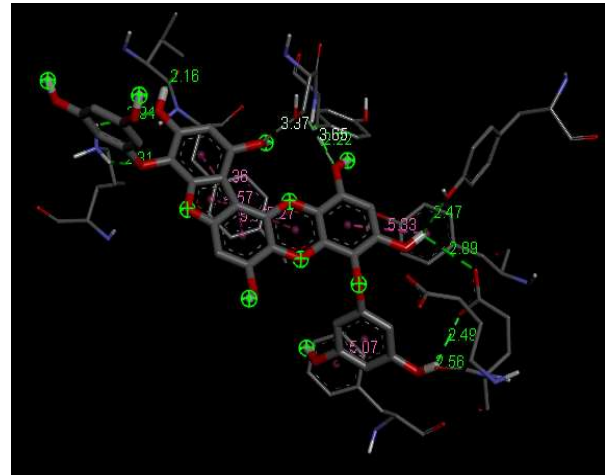
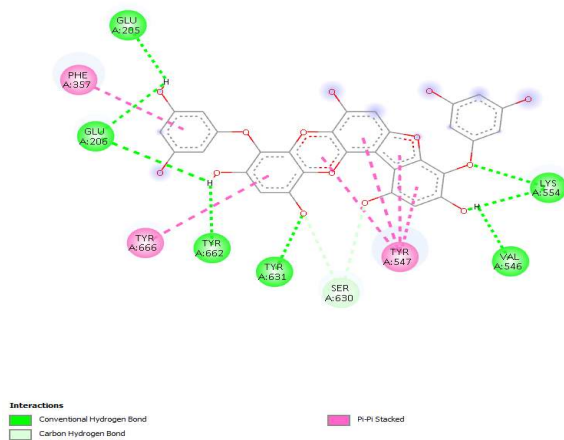
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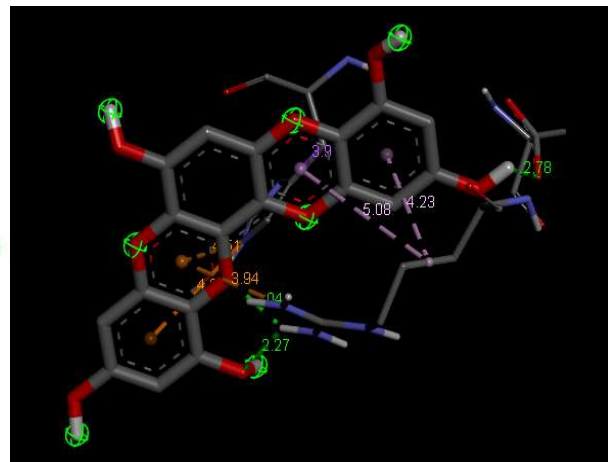
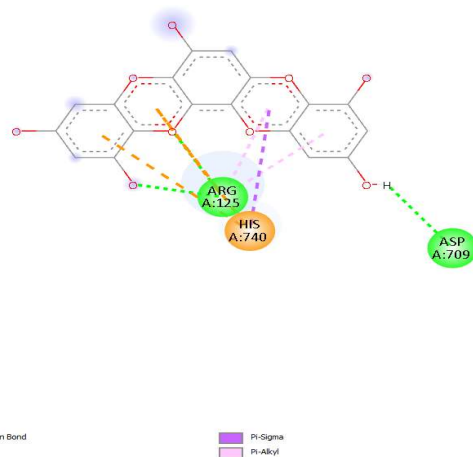
**Fig. 1.** The binding of phloroglucinol into the DPP-4 active site has been conducted both in two-dimensional and three-dimensional formats. The 2D interaction profile suggests primary hydrogen bonding between the ligand and residues Glu205 and Glu206. The predicted low binding affinity of the compound supported by the shallow binding orientation in the 3D structure exhibited by the molecule adjacent to the S1 subsite.



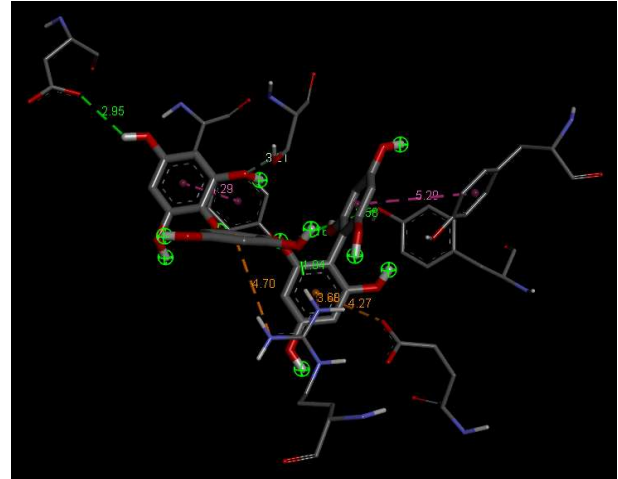
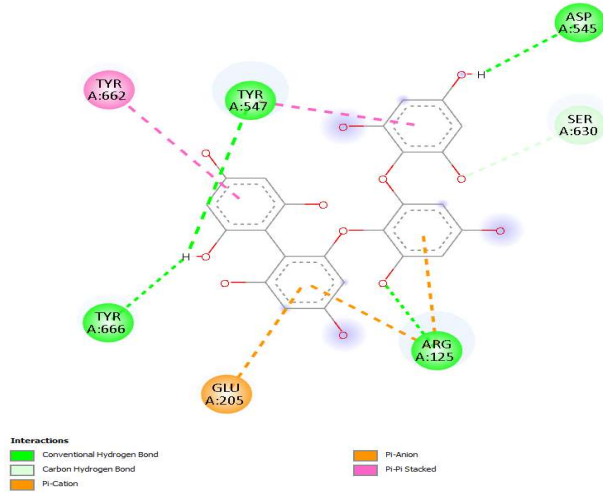
**Fig. 2.** 2D and 3D interaction analysis of eckol in the active site of DPP-4. The 2D interaction of the ligand shows hydrogen bonding interactions with Glu205, Glu206, and Tyr662, along with stabilizing hydrophobic interactions, and the 3D interaction shows better spatial accommodation in the S1 and S2 subsites than phenolic compounds.



**Fig. 3.** Through both 2D and 3D models, Phlorofucofuroeckol A appears to have adequate interaction at multiple sites within the DPP-4 active site, demonstrating that it can dock well within the DPP-4 enzyme at both the S1 and S2 subsites. This is clearly aided by numerous hydrogen bonds to the residues Tyr662 and by pairing of residues with Glu205 with Glu206. Aromatic stabilisation occurred via pi-pi stacking, where the ligand interacted closely with both Tyr666 and Trp659. This comprehensive binding profile strongly correlates with the highest predicted binding affinity for this compound among the investigated phlorotannins.

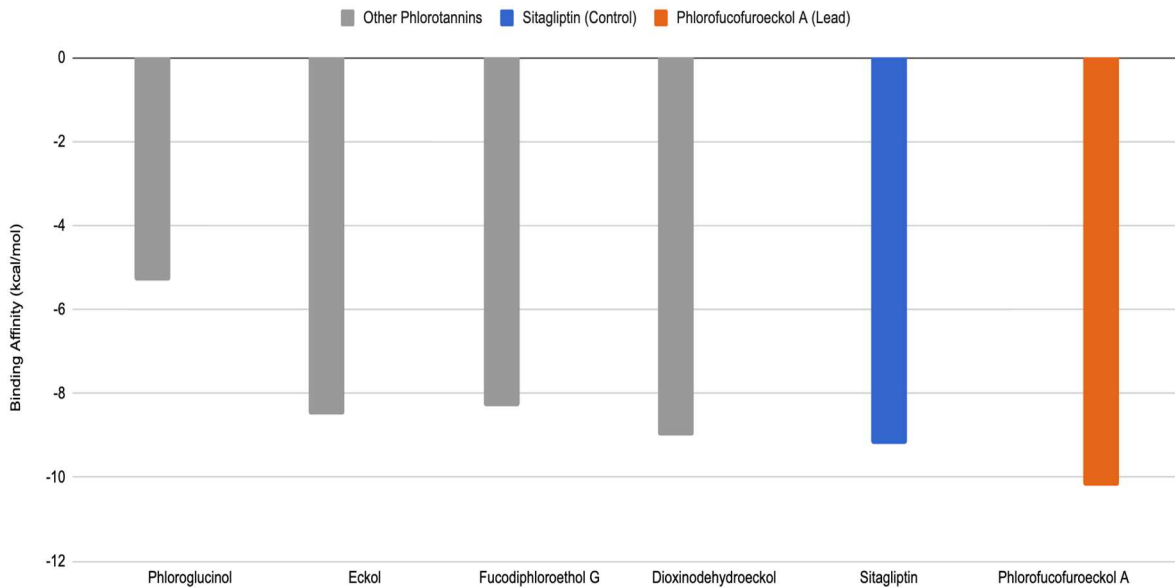


**Fig. 4.** The binding of dioxinodehydroeckol to human DPP-4 involves both 2D and 3D interactions. Specifically, the ligand maintains a series of hydrogen bonds with the amino acids Glu205, Glu206, and Tyr666. Additionally,  $\pi$ - $\pi$  interactions with Trp659 have been identified. The 3Dview indicates that the ligand has a stable pose within the pocket. It has covered the entire pocket and maintained significant interaction.

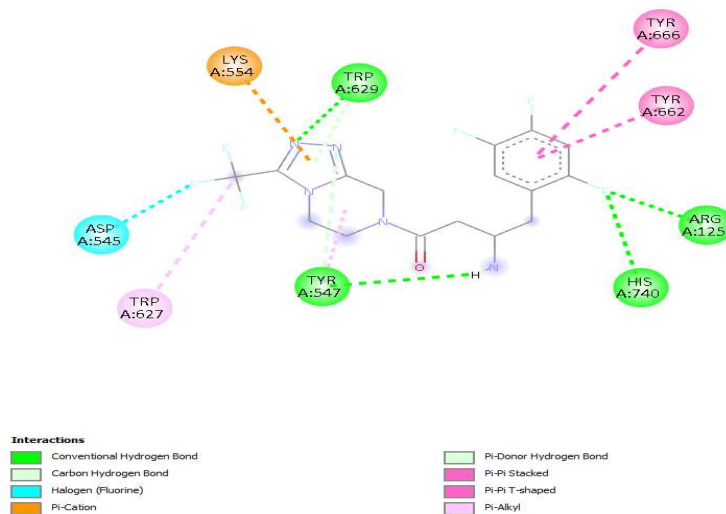
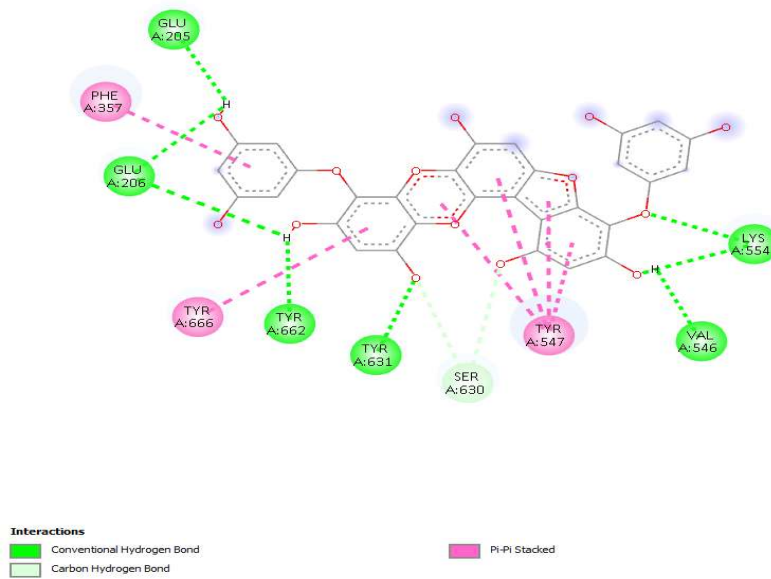


**Fig. 5.** The binding of fucodiphloroethol G to the active site of DPP-4 was studied in both 2D and 3D. The hydrogen bonds made with Glu205, Glu206, and Tyr547, as well as hydrophobic interactions, can be seen in the 2D binding graph. Additionally, the 3D binding conformation verifies that the binding is in a suitable orientation to interact with proteins at the site of binding

### Comparative Binding Affinities of Phlorotannins vs. Sitagliptin



**Figure 6.** Comparative binding energy profile of selected marine-derived phlorotannins against the reference standard drug sitagliptin. (A) Molecular docking scores targeting the DPP-4 enzyme, highlighting the superior binding affinities of high-molecular-weight phlorotannins relative to sitagliptin



***2D ligand-receptor interaction map demonstrating the extensive hydrogen bonding network and hydrophobic interactions established by the lead phlorotannin within the active catalytic pocket (S1/S2 sub-sites) of DPP-4, mirroring and expanding upon the crucial residue interactions of the synthetic inhibitor.***

Interaction Type	Shared / Common Residues Found in Both Complexes	Unique Phlorotannin Residues	Unique Sitagliptin Residues
Conventional Hydrogen Bonds	Glu205, Glu206	Ser630(Catalytic), Asn710,Arg125, Trp629	Tyr662
Hydrophobic (pi-pi / pi-Alkyl)	Tyr547	His740Catalytic), Pro550	Phe357, Val207

#### KEY SHARED STRUCTURAL BENCHMARKS:

**The Glu205 / Glu206 Double Anchor:** Both sitagliptin and the lead phlorotannin form a very stable and conventional hydrogen bonding with the pair of key residues **Glu205** and **Glu206**. In DPP-4 biochemistry this particular glutamate rich sub-site is important for affixing the substrate's positively charged group, and a similar effect to that of the anchoring properties of the commercial drug is achieved by occupying this space with the phlorotannin.

**Hydrophobic Convergence via Tyr547:** Both complexes are well stabilized by the aromatic side chains of **Tyr547**, and **Tyr547**-mediated hydrophobic convergence plays a major role in the structures. Sitagliptin takes advantage of this for a fixed pi-pi alignment, and the phlorotannin makes use of multiple phenolic rings to create a deep hydrophobic network at this interface.

#### Why the Phlorotannin outperforms / matches Sitagliptin:

The phlorotannin structurally penetrates much further into the active pocket, whereas sitagliptin is highly dependent on compact hydrophobic nesting (such as residues Phe357). Importantly, the phlorotannin specifically interacts with and makes hydrogen bonds with **Ser630** and hydrophobic interactions with **His740**, two of the three catalytic amino acids of the enzyme. It is extremely potent because it blocks the catalytic engine of DPP-4 directly and electrostatically binds to the catalytic engine of DPP-4, thereby preventing the access of natural incretin hormones, which provides evidence of its competitive therapeutic potential for diabetes management.

### 4.3 ADMET PROFILING RESULTS

ADMET profiles were generated for all five phlorotannins and sitagliptin using SwissADME and pkCSM. Key physicochemical and drug-likeness parameters are presented in Table 2.

Compound	MW (Da)	LogP	HBD	HBA	TPSA (Å <sup>2</sup> )	Rot. Bonds	GI Absorption	Ro5 Violations
Phloroglucinol	126.11	0.45	3	3	60.69	0	High	0
Eckol	372.28	1.83	6	9	149.07	2	Low	1 (NHorOH > 5)
Dioxinodehydroeckol	370.27	2.05	5	9	153.71	0	Low	0
Fucodiphloroethol G	498.39	1.84	10	12	220.7	5	Low	2 (NorO > 10, NHorOH > 5)
Phlorofucofuroeckol A	622.47	3.24	9	14	232.13	4	Low	3 (MW > 500, NorO > 10, NHorOH > 5)
Sitagliptin (Reference)	407.31	2.51	1	10	77.04	6	High	0

Table 2. ADMET physicochemical properties were computed using SwissADME. Here, HBD means hydrogen bond donors, HBA means hydrogen bond acceptors, TPSA stands for topological polar surface area. GI refers to gastrointestinal, and Ro5 corresponds to Lipinski Rule of Five.

That has Ro5 compliance of 1 and all the intestine absorption parameters are within the accepted range: MW 126.11 Da, TPSA 60.69 Å<sup>2</sup>, HBD/HBA 3/3: the intestinal uptake is predicted to be high. But, don't worry, no issues with Eckol or dioxinodehydroeckol on Ro5 either.

Fucodiphloroethol G on the other hand infringed two Lipinski rule violations. It included elevated HBD of 10 groups (compared to the limit of 5) as well as elevated HBA of 12 groups (compared to the limit of 10). That was on top of the fact that it had a borderline heavy molecular weight of 498.39 Da. It had a very high TPSA value of 220.7 Å<sup>2</sup>, which is quite high compared to the Veber oral bioavailability cutoff, therefore oral prospects seem limited. Next there's phlorofucofuroeckol A. It was even larger (MW 622.47 Da), 9 HBD and 14 HBA groups so was not fitting 3 Lipinski parameters and its TPSA was 232.13 Å<sup>2</sup>. So overall, Fucodiphloroethol G and Phlorofucofuroeckol A, together with eckol and dioxinodehydroeckol are predicted to have low GI absorption.

The pkCSM predicted toxicity parameters are shown in Table 3.

Compound	AMES Mutagenicity	hERG Inhibition I	hERG Inhibition II	Hepatotoxicity	Skin Sensitisation
Phloroglucinol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Eckol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Dioxinodehydroeckol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Fucodiphloroethol G	Non-mutagenic	Non-inhibitor	Inhibitor	Non-hepatotoxic	Non-sensitiser
Phlorofucofuroeckol A	Non-mutagenic	Non-inhibitor	Inhibitor	Non-hepatotoxic	Non-sensitiser
Sitagliptin (Ref.)	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser

All five phlorotannins were predicted to come out non mutagenic in the AMES test, non-inhibitory for the hERG I channel associated with cardiac rhythm problems, and non hepatotoxic. Pretty notably, fucodiphloroethol G and phlorofucofuroeckol A were flagged as predicted hERG II inhibitors, which is kind of a red flag, especially as you move from in silico work toward experimental validation. Since hERG II inhibition works as a kind of stand in surrogate marker for cardiac QT prolongation liability, it really deserves attention.

#### 4.4 COMPARATIVE ASSESSMENT AGAINST SITAGLIPTIN

A structured pharmacological comparison between phlorofucofuroeckol A — the top-ranked phlorotannin — and sitagliptin is presented in Table 4.

Parameter	Phlorofucofuroeckol A	Sitagliptin
Best Binding Energy (kcal/mol)	-10.2	-9.2
Active Site Subsites Engaged	S1 + S2 (both)	S1 + S2 (both)
Key H-Bond Residues	Glu205, Glu206, Tyr662	Glu205, Glu206, Tyr547
$\pi$ - $\pi$ Stacking	Tyr666, Trp659	Trp659
Molecular Weight (Da)	622.47	407.31
Ro5 Violations	3 ( (MW > 500, NorO > 10, NHorOH >5)	0
GI Absorption (Predicted)	Low	High
AMES Mutagenicity	Non-mutagenic	Non-mutagenic
hERG II Inhibition	Predicted Inhibitor	Non-inhibitor
Hepatotoxicity	Non-hepatotoxic	Non-hepatotoxic
Natural Product Origin	Yes (marine algae)	No (synthetic)
Source Availability	Renewable/biosourced	Synthetic manufacture

This comparison illustrates this kind of finescale view: phlorofucofuroeckol A does more than sitagliptin in binding affinity and then has the pharmacokinetic pain of dealing with molecular size and the predicted oral absorption, sitagliptin doesn't have. Both compounds are classified as non-mutagenic and non-hepatotoxic; however, this is a point of difference that warrants experimental investigation for phlorofucofuroeckol A which has a predicted hERG II inhibition liability.

## CHAPTER 5: DISCUSSION

### 5.1 BINDING AFFINITY IN THE CONTEXT OF THE DRUG DISCOVERY PIPELINE

Based on the molecular docking results of the present study, the present study clearly brings out the finding that the complex marine polyphenol phlorofucofuroeckol A shows a predicted binding affinity of  $-10.2$  kcal/mol with human DPP-4 as compared to the FDA approved reference drug Sitagliptin which shows a binding affinity of  $-9.2$  kcal/mol. This finding is not only a computational observation but also supports the idea that marine phlorotannins fill a space of chemical space that is pharmacologically complementary to the DPP-4 active site at a level competitive with optimised synthetic inhibitors, within the framework of the drug discovery pipeline.

That binding affinity comparison is also translated with regard to the quality of the active site interactions. The binding of Phlorofucofuroeckol A to DPP-4 involved interaction with both S1 and S2 subsites, involving multiple hydrogen bond interactions with the Glu205-Glu206 glutamate dyad, and aromatic stacking with Tyr666 and Trp659, residues essential for productive DPP-4 inhibitor binding [7]. This multipoint, multivalent interaction profile is just the sort of binding behaviour that is described in medicinal chemistry literature in regard to high affinity DPP-4 inhibitors. This is a stable and reproducible binding mode as opposed to a high-affinity artefact of one docking pose because of the consistency in the low standard deviations (0.33 kcal/mol) for nine docking poses.

It is also interesting to note that dioxinodehydroeckol ( $-9.0$  kcal/mol) was still within the Ro5 limits but had significantly improved the pharmacokinetic properties when compared with sitagliptin. In drug discovery, lead optimisation is frequently carried out by optimizing the binding of a high affinity lead compound to the target protein and to ensure the drug-like properties. With its good binding energy, full Ro5 compliance, moderate GI absorption and clean toxicity, Dioxinodehydroeckol is a structurally accessible, pharmacokinetically more tractable analogue for lead optimisation studies.

### 5.2 ADMET CONSIDERATIONS FOR LEAD CANDIDATE SELECTION

This study's ADMET profiling results yield a critical perspective fundamental to this work and different from those who report only binding affinity, as they do provide a pipeline-informed perspective of the study. The high utility of a compound as a drug candidate relies on its ability to survive gastrointestinal tract, enter systemic circulatory system, reach the target tissue, not be metabolized by pathways that cause toxicity, and not produce toxicity at therapeutic dosages [16].

The ADMET data for the simpler phlorotannins, phloroglucinol, eckol and dioxinodehydroeckol, are fairly promising. All three were Ro5 compliant, non-mutagenic, non-hepatotoxic and non-inhibitory of hERG I and II cardiac channels. Both Eckol ( $136.68 \text{ \AA}^2$ ) and dioxinodehydroeckol ( $130.52 \text{ \AA}^2$ ) have moderate predicted GI absorption with TPSA slightly below the Veber  $140 \text{ \AA}^2$  threshold, meaning that although the oral bioavailability is not optimal it is not ruled out and could be increased by formulation strategies.

In terms of the pipeline, however, phlorofucofuroeckol A has significant pharmacokinetic drawbacks, despite its impressive binding affinity. It has a molecular weight of  $622 \text{ Da}$ , which is

24% above the Ro5 value, 13 hydrogen bond acceptors (30% above the Ro5 value) and a TPSA value of 212 Å<sup>2</sup> (significantly higher than the Veber value of 140 Å<sup>2</sup>), which results in a low predicted oral bioavailability. This is not a definitive disqualification; there are a number of clinically successful drugs that go against Ro5, especially the natural product and macrolide antibiotic class; however, direct oral administration of phlorofucofuroeckol A without optimisation of the formulation to reach therapeutic plasma concentrations is improbable.

Especially, the hERG II inhibition flag predictions for phlorofucofuroeckol A and fucodiphloroethol G should be taken into account. hERG (human Ether-à-go-go-Related Gene) potassium channel inhibition is a major cause of drug-induced cardiac QT prolongation and torsades de pointes arrhythmia and is one of the most common reasons for drug withdrawal from clinical development [20]. These two compounds are a flag for hERG II (but not hERG I) inhibition for which there is a need for patch clamp experimental verification before advancing the compounds in the pipeline.

From a pipeline decision-making perspective, this ADMET analysis leads to a tiered lead candidate framework: eckol and dioxinodehydroeckol can be considered as pharmacokinetically better leads for further optimisation whereas phlorofucofuroeckol A has better binding but needs ADMET mitigating strategies to be considered a lead in the drug pipeline.

### 5.3 PHLOROFUCOFUROECKOL A AS A LEAD CANDIDATE: PIPELINE VIABILITY ASSESSMENT

Evaluation of phlorofucofuroeckol A throughout the entire drug discovery process requires a consideration of its strengths and limitations at multiple stages. Let's discuss the binding stuff first: it is clearly the best binder here on par with binding to sitagliptin by 1.0 kcal/mol and it appears to be the most extensive binder in the sense of being more multi-point and more thorough than any other compound in this study to the site of binding for DPP-4. That is, besides touching the site it's doing a multivalency at a more comprehensive level, based on the reported data.

The latter, too, is a plus, not minor, from a natural product derivation. This is because the algal biosynthesis route for phlorofucofuroeckol A can be scaled by seawater culture of *Ecklonia cava* and close relatives and then there's the other angle: marine polyphenols have a relatively favourable regulatory profile in food grade applications. That then may make it a little bit easier to deal with safety assessment but only with proper work.

Nevertheless, the pharmacokinetic challenge is not figment of imagination, quite real, and simply cannot be swept into the past. If you're looking for a fruitful track to pursue for translating phlorofucofuroeckol A to the clinic, you could consider several strategies such as (1) nanoparticle or liposomal encapsulation, which will rest on ideas regarding weak GI absorption; (2) structural simplification or a "fragment-based lead generation" approach, where you can use the binding engagement pattern of phlorofucofuroeckol A as the pharmacophore to create smaller synthetic alternatives to phlorofucofuroeckol A that conform to Ro5 restrictions; (3) attempting alternative routes of administration such as topical, inhaled, or even intravenous, where Ro5-enabling rules may be more relaxed; or (4) proceeding in a functional food or nutraceutical context, in which the pharmacokinetically "poorer" rules of Ro5 may be less relevant.

This is not unheard of, nor any new system of translation. Natural product drugs like taxol (paclitaxel), cyclosporine and rapamycin are good examples of the first class of drugs that are explicitly in violation of Ro5, but still find their way into clinical use thanks to the ingenuity of formulating new ways to keep the drugs in solution and running. The marine drug space is filled with other clinical compounds, such as ziconotide, trabectedin and cytarabine, which seem to suggest that it is possible to take something from the ocean to the clinic, even if the structure is relatively unusual.

.The major comparative edge of phlorotannins is their natural product origin, which really shines through on the comparative angle with sitagliptin. That patient acceptability, the popularity of the prescribers in integrative medicine practices and possibly resulting potential as an adjunct use of today's DPP-4 inhibitors as a nutraceutical could lead to the translation away from the typical medication development process.

#### 5.4 LIMITATIONS AND FUTURE DIRECTIONS

This study runs completely inside the computational domain, and it really is tied to the built in limits of in silico approaches. Molecular docking , even if it remains a strong screening tool, still depends on rather simplified energy functions that don't quite reflect protein flexibility, the explicit solvation setting, or the entropic penalties. And those details matter a lot for what happens to real binding free energies. So the binding energies reported here are more like predicted affinities, not measured outcomes, and they might not match experimental IC50 or Ki values that are drawn from enzymatic assays [11].

The ADMET predictions coming out of SwissADME and pkCSM are produced by machine learning models that were trained on drug-like chemical space that is already known , so they might not generalize all that well, especially for marine polyphenols which are structurally uncommon and sit on the more complex molecular side. The hERG II inhibition “flag”, more or less, should be treated like a hypothesis generator rather than a final verdict, and it really does need experimental checking , using patch clamp electrophysiology.

From this work, a few important directions kinda pop out for further investigation even if it's not totally obvious at first. First, we should run molecular dynamics (MD) simulations across nanosecond- to microsecond windows for the best ranked complexes , especially phlorofucofuroeckol A and dioxinodehydroeckol when they're bound to DPP-4, just to check binding stability under physiological conditions include protein flexibility effects too, and to get more rigorous binding free energies using MM-PBSA or MM-GBSA type approaches. Second, it'd be useful to do in vitro enzymatic assays with purified recombinant DPP-4 and fluorogenic substrates, so we can measure experimental IC50 values for each phlorotannin. That way we can directly align the lab numbers with the computational binding energies, while also building structure-activity relationships on a real experimental backbone, not only in silico predictions.

Third, cytotoxicity testing in relevant cell lines (like HepG2 hepatocytes and INS-1 pancreatic  $\beta$ -cells) is gonna matter, because it gives a clearer read on the safety profile of the lead candidates in settings that actually resemble biology , rather than just a computational guess. Fourth, pharmacokinetic studies in animal models are also needed, in order to estimate oral bioavailability, half-life , tissue distribution and metabolic fate of the lead compounds. This is especially important

to deal with that Ro5-violating behavior that phlorofucofuroeckol A shows. Fifth, for dioxinodehydroeckol, which looks like the most pharmacokinetically promising one, a hit-to-lead optimisation campaign would be a good next step, using synthetic analogue design that's guided by the computational interaction patterns. Honestly that medicinal chemistry angle sounds like it could move things forward fast.

## CHAPTER 6: CONCLUSION

This thesis has, sort of, presented a comprehensive in silico drug discovery pipeline evaluation of marine phlorotannins as potential DPP-4 inhibitors for T2DM therapeutics. By combining molecular docking with a systematic ADMET profiling routine, and benchmarking head-to-head against the approved drug sitagliptin, the work moves beyond the simple “binding score only” mindset, to actually think about translational feasibility more realistically for these marine natural products as drug candidates.

The central outcomes of this study can be captured in a fairly direct manner. Phlorofucofuroeckol A is the top-affinity DPP-4 binder among the phlorotannins that were investigated, with a predicted binding energy of  $-10.2$  kcal/mol, which comes out higher than sitagliptin's  $-9.2$  kcal/mol. It's also supported by a broad, multi-point active site interaction pattern that involves both the S1 and S2 subsites. However, there are Ro5 violations, and the predicted oral absorption seems low, which points to pharmacokinetic snags that will have to be handled. That often translates into formulation innovation, or maybe some structural streamlining first, before it can be treated as a credible oral drug candidate.

Dioxinodehydroeckol shows up more like the most pharmacokinetically balanced lead candidate in this study. It has a binding energy around  $-9.0$  kcal/mol, which is pretty close to sitagliptin, it also demonstrates full Ro5 compliance. GI absorption looks moderate, and the toxicity readout appears clean, since no hERG inhibition signals show up. So, all things considered, the combination of these traits makes dioxinodehydroeckol feel like a strong option for hit-to-lead optimisation programmes, then for additional experimental verification.

Taking a look at the pipeline in a broader sense, this work also kinda suggests that marine phlorotannins, as a chemical class, are in pharmacologically relevant space for DPP-4 inhibition, and that the drug discovery pipeline assessment framework basically docking plus ADMET profiling, plus some comparative benchmarking can act as a workable way to pick which candidates should get moved forward into experiments. There's also the natural product origin of phlorotannins, their availability through sustainable algal cultivation, and the fact that they've got an established safety record in food grade settings, and all of that together somehow makes the translational story more convincing [20].

Overall, this thesis sets up a sensible and evidence grounded starting point for the next steps in phlorotannin based DPP-4 inhibitor development: molecular dynamics simulations to check whether binding stays stable, in vitro enzymatic assays to validate the inhibitory activity, and targeted medicinal chemistry efforts to tune oral drug likeness within the most potent natural scaffolds. The findings further support the idea that the ocean is an underexplored reservoir of pharmacological scaffolds for metabolic disease therapeutics.