INSTITUTO POLITÉCNICO DE BRAGANÇA Escola Superior Agrária

Study of the anti-diabetic potential of a Fungi Marine Compounds library by virtual screening against 11β-HSD1

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List of abbreviations:

11β-HSD1 – 11β-Hydroxysteroid dehydrogenase type 1

11β-HSD2 – 11β-Hydroxysteroid dehydrogenase type 2

Ala – Alanine

Asn– Asparagine

BMI – High body mass index

CAP – Cbl associated protein

Cbl – Casitas B-lineage lymphoma

CBO – Carbenoxolone

DPP-4 – Dipeptidyl peptidase 4

CKD – Chronic kidney disease

eNOS – Endothelial nitric oxide synthase

FFA – Free fatty acids

G6Pase – Glucose 6-phosphatase

GA – Glycyrrhizinic acid

GCs – Glucocorticoids

GLD – Glucose-6-phosphatase catalytic subunit

GLP-1 – Glucagon-like peptide-1

H1-12 – 12 helices

HbA1c – Hemoglobin A1C

HDL – High-density lipoprotein

HFD – High-fat diet

HOMA-IR – Homeostatic Model Assessment for Insulin Resistance

HTS – High-throughput screening

IRS – Insulin receptor substrate

Ile – Isoleucine

Km – Michaelis-Menten constant

LDL – Low-density lipoproteins

LFD – Low-fat diet

Lys – Lysine

MR – Mineralocorticoid

NADPH – Nicotinamide adenine dinucleotide phosphate

NAFLD – Nonalcoholic fatty liver disease

NDA – New Drug Application

NLRP3 – Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3

p-AKT – Phosphorylated Akt

PEPCK – Phosphoenolpyruvate carboxykinase

PHA – Pseudohypoaldosteronism type I

IκB-α – Inhibitor of nuclear factor kappa B

NF-κB – Nuclear factor kappa B

PI3K – Phosphoinositide 3-kinase

NMR– Nuclear Magnetic Resonance

S1-7 – Seven chains

SBVS – Structure-based virtual screening

Ser– Serine

SGLT2 – Sodium-glucose cotransporter-2

T2DM – Type 2 diabetes mellitus

Thyr – Tyrosine

Tyr – tyrosine

VS – Virtual Screening

Val – Valine

Abstract:

Type 2 diabetes mellitus (DM) is a chronic metabolic disease whose prevalence has been steadily increasing worldwide. Type 2 DM (formerly known as non-insulin-dependent DM) is the most common form of DM, characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. This metabolic disease results from the interaction between genetic, environmental and behavioral risk factors. One of the proteins involved in this disease is 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme responsible for the reduction of cortisone to its active form, cortisol, which can lead to metabolic alterations such as insulin resistance and hyperglycemia. Thus, inhibition of 11β-HSD1 may offer a novel therapeutic approach for type 2 diabetes mellitus. Marine fungi (MF) compounds have been the subject of great attention in drug discovery because of their promise as therapeutic agents and because they possess a range of activities, including antibacterial, antiviral, and anticancer agents. This study prepared a virtual library of 157 compounds in marine fungi (MF). The potential of the compounds in the MF library as inhibitors of the 11β-HSD1 was then evaluated. Molecular docking of the MF library was performed against the 3D structure of 11β-HSD1 using VINA and YASARA software. Out of the 157 compounds, the 3 compounds that presented lower predicted binding energies (ΔG) values were Brevione A, Brevione I and Ilicicolin H with ΔG values of -11.6, -11.4 and -10.9 (kcal/mol), respectively. Brevione A and Brevione I are present in marine fungi *Penicillium sp,* while Ilicicolin H is present in *Campylocarpon* sp. HDN13-308. A detailed analysis of the predicted binding conformation of the 3 compounds was performed, and the main residues involved in the predicted binding conformation were analyzed in detail. Although experimental validation is needed, the 3 highlighted compounds, especially Brevione I, can be considered promising leads for developing potential anti-diabetic therapeutics. Also, the MF library prepared will be made available for researchers in general and will eventually be used to virtually screen other protein targets of interest.

Keywords: Diabetes, Marine Fungi; Virtual Screening; 11β-HSD1

Resumo:

O diabetes mellitus tipo 2 (DM) é uma doença metabólica crónica cuja prevalência tem estado em aumento constante em todo o mundo. O DM tipo 2 (anteriormente conhecido como DM não dependente de insulina) é a forma mais comum de DM, caracterizada por hiperglicemia, resistência à insulina e deficiência relativa de insulina. Esta doença metabólica resulta da interação entre fatores de risco genéticos, ambientais e comportamentais. Uma das proteínas envolvidas nesta doença é a 11β-hidroxiesteróide desidrogenase tipo 1 (11β-HSD1), uma enzima responsável pela redução da cortisona à sua forma ativa, o cortisol, que pode levar a alterações metabólicas como resistência à insulina e hiperglicemia. Assim, a inibição da 11β-HSD1 pode oferecer uma nova abordagem terapêutica para a DM tipo 2. Os compostos de fungos marinhos (MF) têm sido objeto de grande atenção na descoberta de medicamentos devido à sua promessa como agentes terapêuticos e porque possuem uma gama de atividades, incluindo antibacteriana, antiviral e antitumoral. Este estudo preparou uma biblioteca virtual de 157 compostos de fungos marinhos (MF – Marine Fungi). O potencial dos compostos da biblioteca de MF como inibidores da 11β-HSD1 foi então avaliado. O docking molecular da biblioteca de MF foi realizado contra a estrutura 3D da 11β-HSD1, utilizando os softwares VINA e YASARA. Dos 157 compostos, os 3 compostos que apresentaram menores valores de energias de ligação prevista (ΔG) foram os compostos Brevione A, Brevione I e Ilicicolin H, com valores de ΔG de -11,6, -11,4 e -10,9 (kcal/mol), respetivamente. O Brevione A e o Brevione I estão presentes em fungos marinhos do género Penicillium sp, enquanto o Ilicicolin H está presente em Campylocarpon sp. HDN13-308. Foi realizada uma análise detalhada da conformação de ligação prevista dos 3 compostos, e os principais resíduos envolvidos nessa conformação de ligação foram analisados em detalhe. Embora seja necessária validação experimental, os 3 compostos destacados, especialmente o Brevione I, podem ser considerados promissores para o desenvolvimento de potenciais novos fármacos antidiabéticos. Além disso, a biblioteca de MF preparada será disponibilizada para investigadores em geral e poderá ser utilizada para procurar potenciais inibidores de outros alvos proteicos de interesse.

Palavras-chave: Diabetes, Fungos marinhos; Seleção virtual; 11β-HSD1

1. Introduction

1.1 Diabetes mellitus

Diabetes mellitus (DM) is a widespread chronic disease that affects glucose metabolism and has serious clinical consequences. The complications of diabetes affect multiple systems and can lead to microvascular issues such as retinopathy, nephropathy, and neuropathy, as well as macrovascular problems like ischemic heart disease, stroke, and peripheral vascular disease. The incidence of diabetes has been increasing in recent decades, largely due to the rise in obesity. The premature morbidity, mortality, reduced life expectancy, and financial burdens on patients with diabetes, their caregivers, and the healthcare system make it a significant public health concern [1].

Diabetes can broadly be classified into three categories: type 1 diabetes or insulin-dependent diabetes accounts for 5–10% of all diabetic cases and is due to autoimmune destruction of the insulin-secreting beta cells in the pancreas; type 2 diabetes or non-insulin-dependent diabetes accounts for 90–95% of diabetic cases and is caused by a combination of beta cell loss and dysfunction together with insulin resistance (IR) in target tissues and finally gestational diabetes mellitus (GDM) that is a form of hyperglycemia identified during pregnancy. GDM is currently the most common medical complication during pregnancy, affecting approximately 15% of pregnancies worldwide [2] [3]. According to [4]in 1995, approximately 135 million people were affected by diabetes, and an increase of 300 million cases was estimated by the year 2025 (Figure 1). In 2011, there were already 336 million people with diabetes, which led to a sudden change in statistics. The new estimates forecast approximately 425 million cases by 2045 [5].

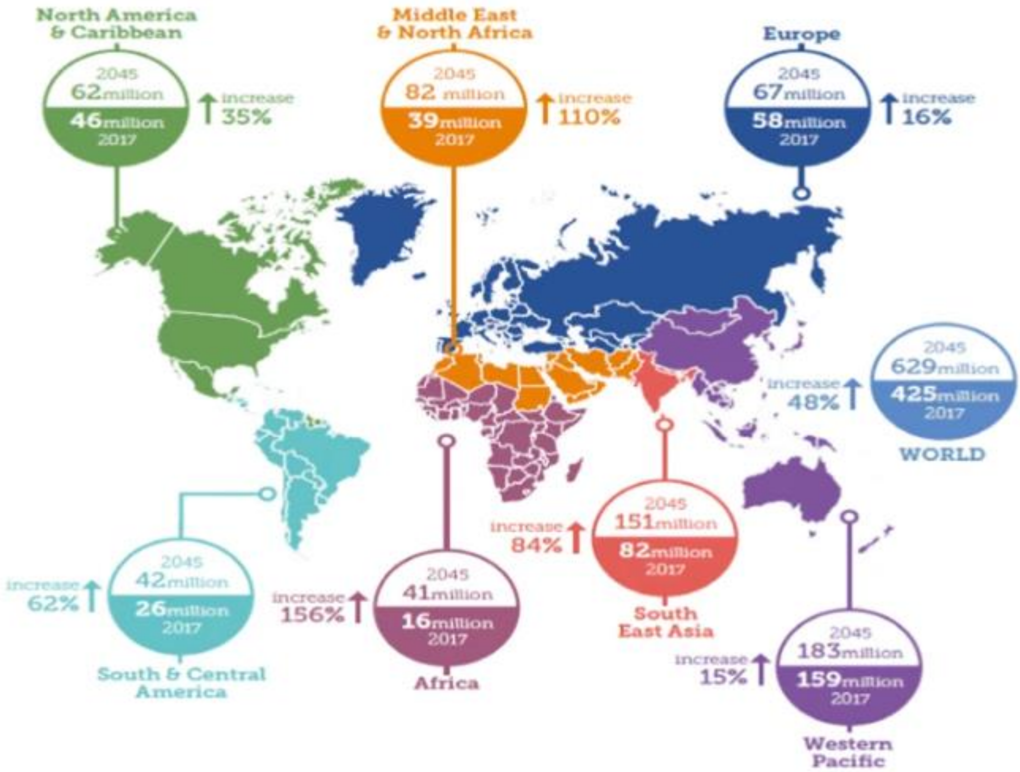


Figure 1- The number of people with diabetes worldwide and by region for 2017 and a forecast for 2045 [5].

Insulin is the core hormone regulating cellular energy supply and macronutrient balance, directing anabolic processes of the fed state [6] In other words, it plays a major role in appropriate tissue development, growth, and maintenance of glucose homeostasis. Insulin is secreted by the pancreatic β cells in response to increased circulating levels of glucose and amino acids after a meal. Insulin regulates glucose homeostasis at several sites, by reducing hepatic glucose production (via decreased gluconeogenesis and glycogenolysis) and increasing the glucose uptake rate, primarily into skeletal muscle and adipose tissue. The response of insulin-dependent cells is only possible due to the binding of insulin to the extracellular portion of the receptor, which activates its kinase activity, resulting in the autophosphorylation of specific intracellular tyrosine residues. This autophosphorylation step enables a variety of scaffolding proteins, including insulin receptor substrate (IRS) proteins, Cbl (casitas B-lineage lymphoma) or Cbl-associated protein (CAP), to bind to intracellular receptor sites and to become phosphorylated [6].

Insulin also affects lipid metabolism, increasing lipid synthesis in liver and fat cells and decreasing fatty acid release from adipose tissue. Insulin resistance is classically defined as a state of reduced responsiveness of target tissues to normal circulating insulin levels;

it plays a major role in developing DM2. Insulin resistance occurs when the cell doesn't up-take glucose effectively, resulting in excess glucose in the bloodstream. Over time, the pancreas keeps trying to regulate the blood sugar, reducing insulin secretion, which causes a reduction in insulin production. As a result, blood sugar levels increase, resulting in a frequent state of hyperglycemia. Insulin resistance is not only caused by a genetic predisposition but is also a feature of several other health disorders, including obesity, glucose intolerance, dyslipidemia and hypertension, which are grouped in the so-called concept of metabolic syndrome (Figure 2) [7].

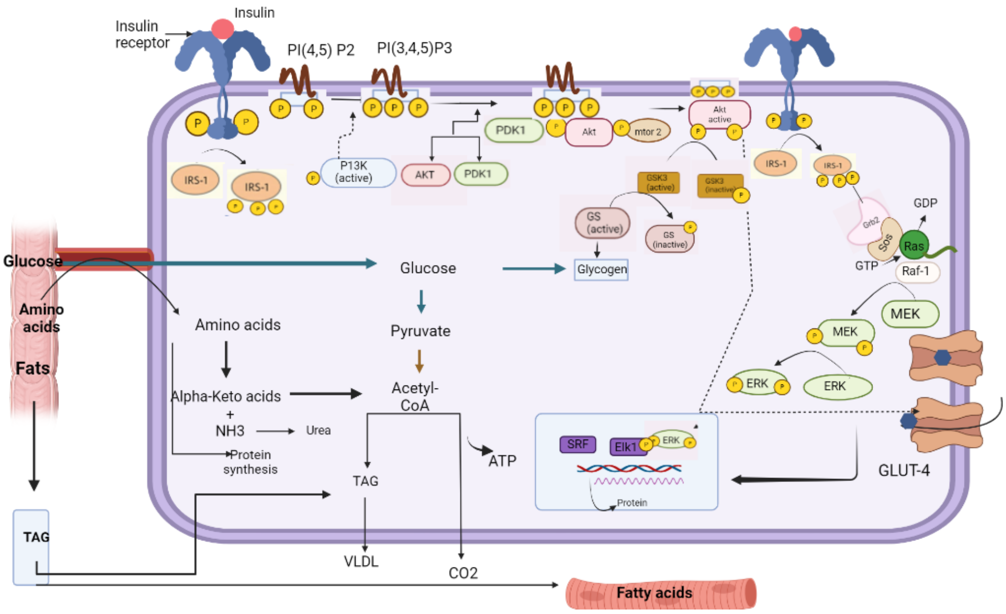


Figure 2- Insulin receptor signalling pathway and its metabolic actions within a cell [9].

1.2 Therapeutic options

Diabetes therapeutic options are several, generally called Glucose-Lowering Treatment (GLD). GLD embraces a wide variety of medicines that have distinct targets in the body with the common goal of reducing blood glucose levels and include several pharmacological treatments: insulin, dipeptidyl peptidase-4 (DPP-4) inhibitors, sulphonylureas, sodium-glucose co-transporter-2 (SGLT2) inhibitors, thiazolidinediones, acarbose or glucagon-like peptide-1 (GLP-1) receptor agonists. Table 1 details each of these types of drugs. However, there is no general consensus on which drug to carbohydratesose, and the focus is on individualized treatment, mainly to improve glucose control [8].

The first step to handle or prevent evolution from prediabetes to T2DM is lifestyle changes concerning dietary habits and exercise that can independently reduce the risk of diabetes. Several studies have shown a lower risk of diabetes with regular exercise, which appears to be greater in individuals at high risk for diabetes, such as those with high body mass index (BMI), hypertension, or family history of diabetes [9]. Furthermore, greater reductions in diabetes risk are noted with higher intensity and duration of activity. In one study, each 500-kcal increase in the weekly energy expenditure reduced the risk of diabetes by 6%. Even non-intensive leisure activities like shopping, ballroom dancing, and playing volleyball may be beneficial in terms of diabetes prevention [10].

Dietary composition is another factor that may guide the progression of diabetes. Different types of food have different effects on postprandial glycemia. The total amount of carbohydrate consumed strongly influences glycemic response. Yet the ideal amount of carbohydrates in the diet is unclear. Most individuals with type 1 or type 2 diabetes in the U.S. report eating moderate amounts of carbohydrates (45% of total energy intake). There are differing opinions in the literature regarding recommendations for low carbohydrates diets in treating diabetes. There have been many studies over the years looking at use of diets with lower carbohydrates content and improvement in blood glucose without detrimental effects [11]. The American Diabetes Association recommends that dietary fiber intake in patients with diabetes should match the recommendations for the general population, to increase the total fiber intake to 14 g per 1000 cal, or 25 g/d for women and 38 g/d for men [12]. Fiber supplements and bulk laxatives are used frequently as additional dietary fiber sources, but since few fiber supplements have been studied for physiological effectiveness, the best advice is to consume foods high in fiber. The Mediterranean-style eating pattern derived from the Mediterranean diet is a good option since it is relatively rich in monosaturated vegetable fats, as it is shown in Figure 3.

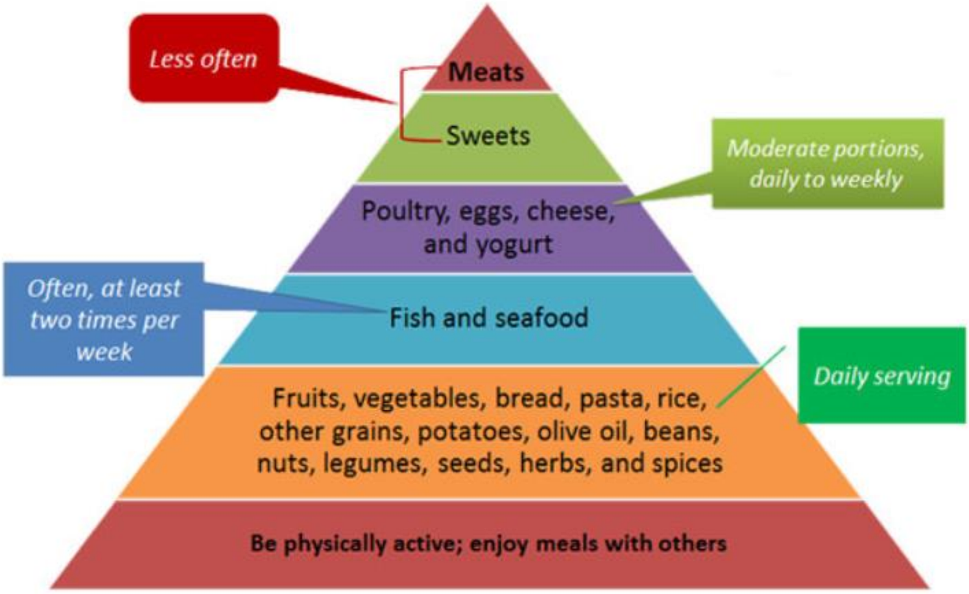


Figure 3- Mediterranean Nutrition [14].

In young patients with a recent diagnosis of T2DM, obesity, or abnormal metabolic profile, one should consider treatment with GLP-1 analogues that would benefit weight loss and improve metabolic dysfunction. GLP-1 analogues are contraindicated in renal failure [13] If the problem persists with dual therapy after 3 months using metformin without glycemic control and HbA1c > 10%, most patients require treatment with basal insulin combined with another agent to achieve the recommended glycemic target. Other options for the treatment of TDM2 are represented in Table 1[14].

Table 1- Pharmacological options for treating Type 2 Diabetes.

Compound	T2DM Action	References
Metformin	Reduces glucose production, best studied and well-tolerated. Used as first-line therapy.	[16] [17]
DPP-4 Inhibitors	Monotherapy after metformin failure for patients not requiring therapy with cardiovascular benefits.	[18]
Sulfonylureas	Add-on therapy promotes insulin secretion. It can cause hypoglycemia and weight gain.	[19]

SGLT2 Inhibitors	Includes dapagliflozin, empagliflozin, canagliflozin. Increases glucose excretion through urine.	[20]
GLP-1 Receptor Agonists	Add-on therapy increases insulin secretion, lowers glucagon release, and reduces appetite.	[20]

1.3 11β-HSD1 and the Metabolic Syndrome

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) plays a crucial role in metabolic syndrome by regulating the conversion of inactive cortisone into active cortisol in the tissues. Increased 11β-HSD1 activity in specific tissues, such as liver and adipose tissue, can lead to elevated local cortisol levels, contributing to various components of the metabolic syndrome, characterized by a large abdominal circumference (due to excessive abdominal fat), high blood pressure, resistance to the effects of insulin (insulin resistance) or diabetes and by altered levels of carbohydrateslesterol and other fats in the blood (dyslipidemia).

1.3.1 11B-HSD and its Influence on Insulin Resistance

11β-HSD1 regulates active cortisol levels in tissues, converting inactive cortisone into active cortisol. High cortisol levels can interfere with insulin signalling, reducing cell glucose uptake and leading to insulin resistance. Inhibiting 11β-HSD1 has been shown to improve insulin sensitivity, highlighting the link between excess cortisol and insulin resistance in metabolic syndrome [17]. Additionally, this inhibition reduces glucocorticoid receptor (GR) signalling, benefiting tissues like the liver and adipose tissue and alleviating metabolic and inflammatory issues. Consequently, 11β-HSD1 inhibition shows promise as a treatment approach for type 2 diabetes, obesity, and metabolic syndrome. While various 11β-HSD1 inhibitors exist, including natural and pharmaceutical compounds like metyrapone, carbenoxolone (CBO), and glycyrrhetinic acid (GE), only selective 11β-HSD1 inhibitors have demonstrated efficacy in diabetic animal models, underscoring their therapeutic potential in metabolic disorders [15].

1.3.2 Importance of 11β-HSD1 in the treatment of DM2

The 11β-HSD1 enzyme is found in various parts of vertebrate species, including liver cells, adipose tissue, pancreatic islet cells, skeletal and cardiac muscle, gonads, inflammatory cells, brain, placenta, and fetus. In rodents, 11β-HSD1 is mainly present in the cerebellum, hippocampus, cortex, and pituitary but can also be found in other brain areas. This enzyme relies on nicotinamide adenine dinucleotide phosphate ($NADP^+$), while 11β-HSD2 relies on nicotinamide adenine dinucleotide (NAD^+) [20].

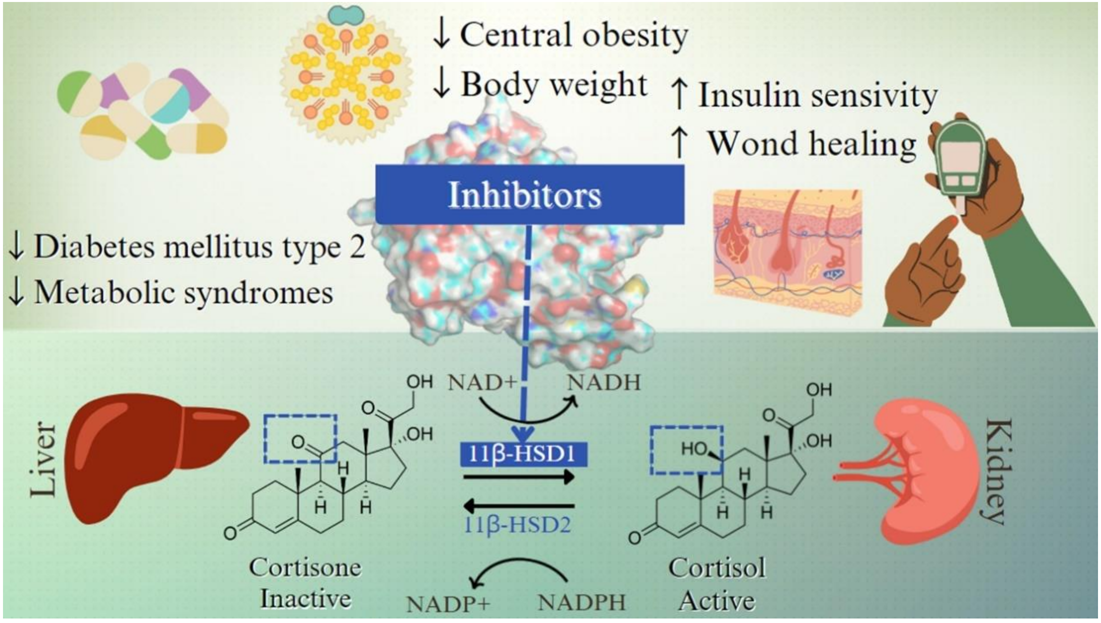


Figure 4- Mechanism of action of 11β-HSD1 inhibitors and their metabolic effects in controlling obesity, type 2 diabetes and metabolic syndromes. Self-authored (2024).

11β-HSD1 converts inactive glucocorticoids, like cortisone, into active forms, such as cortisol, mainly in peripheral tissues. Studies have shown that 11β-HSD1 follows Michaelis-Menten kinetics concerning cortisol but exhibits cooperative kinetics concerning cortisone. This means that the enzyme can function at different substrate concentrations, adapting to changes in glucocorticoid levels. Extensive research has revealed the distinct functions of the 11β-HSD1 and 11β-HSD2 isoenzymes, which are crucial for glucocorticoid metabolism [21][22].

In short, although 11β-HSD1 and 11β-HSD2 share structural similarities in their active sites, they differ in terms of oligomerization and enzyme cofactor requirements. Thus, they play distinct and complementary roles in glucocorticoid metabolism (Figure 5). These differences are critical for the precise regulation of hormonal signalling in target tissues, ensuring the right balance between the activation and deactivation of glucocorticoids [23].

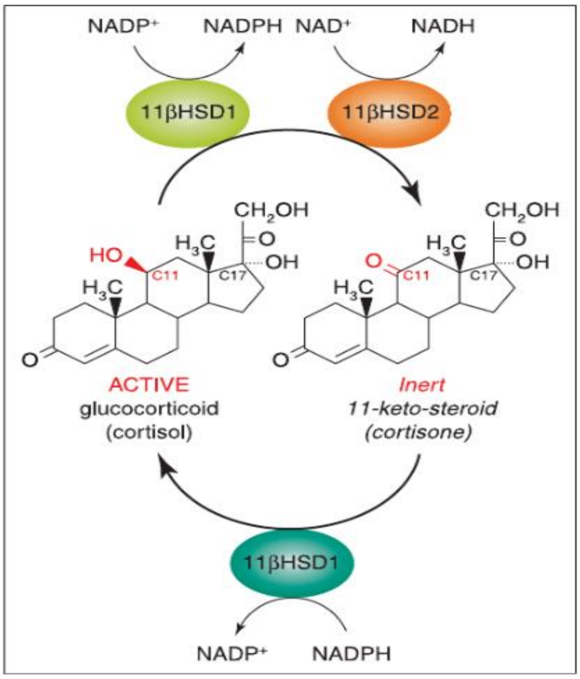


Figure 5- The conversion between cortisol and cortisone occurs through enzymatic oxidation or reduction at the 11-carbon position (C11), catalyzed by the enzyme 11β-HSD1 [20].

Two studies provide complementary evidence regarding the role of 11β-HSD1 in type 2 diabetes mellitus. The first study, conducted in humans, found that increased activity of 11β-HSD1, assessed through factors such as physical exercise, BMI (Body Mass Index), SGOT (Glutamic Oxalacetic Transaminase) levels, and glycemic control—is associated with poorer glycemic control [21]. These results underscore the importance of regulating this enzyme to improve insulin sensitivity and optimize patients' metabolic profiles. In line with these findings, a second study conducted in a mouse model of type 2 diabetes demonstrated that a selective inhibitor, KR-67105, significantly reduced glucose levels and improved other metabolic parameters. Together, these findings suggest that inhibiting 11β-HSD1 may represent a promising therapeutic strategy for treating type 2 diabetes, such as the bisphenol A (BPA) analog bisphenol H (BPH) [23], (2E,6E)-2,6-bis(2-(trifluoromethyl)benzylidene)cyclohexanone (LG13) [24], and the 1,2,4-triazole derivatives used for 11β-HSD1 inhibition [25]. However, these inhibitors are still in experimental and preclinical stages and have not been approved for clinical use in humans [22].

1.3.3 Protein structure of 11β-HSD1

The human 11β-HSD1 structure comprises 292 amino acids and has a molecular mass of approximately 34 kDa. This enzyme has an N-terminal domain that binds to nucleotide cofactors and a catalytic active site in its central region. The N-terminal region specifically binds to the cofactors $NADP^+$ and NADPH. The enzyme's active site contains Tyr183 and Lys187 residues, which interact with the glucocorticoids (GCs) substrates, along with two other essential residues, Asn143 and Ser170. These four amino acids are crucial for proton transfer between the GC substrates and the NADP cofactor [36].

In Figure 6, we can see the overall structure of human 11β-HSD1. This enzyme is part of the short-chain dehydrogenases/reductases family and contains a conserved Rossmann fold for nucleotide binding. The 11β-HSD1 has a parallel β-sheet with seven chains (S1-7) and 12 helices (H1-12).

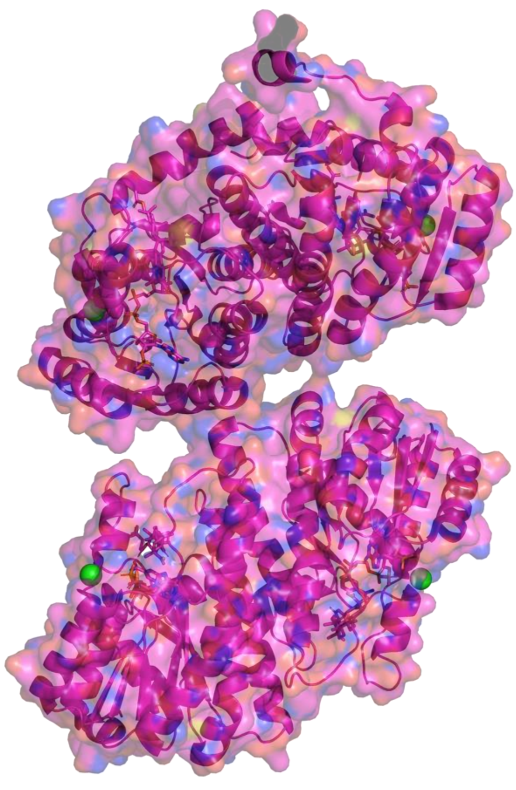


Figure 6- Three-dimensional structure representation of human wild-type 11-beta-hydroxysteroid dehydrogenase (PDB: 2BEL).

1.3.4. Function and expression of 11β-HSD1 in other tissues

New research highlights that enzymes 11β-HSD1 and 11β-HSD2 influence endothelial nitric oxide synthase (eNOS) activity, impacting vascular function. A glucocorticoid response element (GRE) was identified in the eNOS promoter region, where cortisol binds and promotes eNOS expression reduction. This suppression may be linked to conditions like metabolic syndrome or 11β-HSD2 deficiency. Additionally, glucocorticoids affect the central nervous system (CNS), influencing neurotransmission, neuronal growth, metabolism, and survival. Elevated glucocorticoid levels can impair cognitive function, with patients suffering from Cushing's syndrome often experiencing cognitive, psycarbohydratestic, and metabolic issues [25]. Elevated glucocorticoid (GC) levels are associated with cognitive impairments in aging rodents and humans. Studies suggest maintaining low GC levels throughout life may help prevent age-related cognitive decline. 11β-HSD1 is present in the brain, especially in regions like the cerebellum, hippocampus, and neocortex, indicating its potential role in memory and learning processes [26]. Studies confirm the presence of 11β-HSD1 in specific brain regions, while 11β-HSD2 is absent. This raises the possibility that 11β-HSD1 inhibition could be a therapeutic strategy for treating cognitive deficits. 11β-HSD1 produces active glucocorticoids (GC), which may enhance neurotoxicity caused by excitatory amino acids. Additionally, aged 11β-HSD1-/- mice show resistance to cognitive decline, suggesting that inhibiting 11β-HSD1 could offer neuroprotective benefits [27][28]. Studies indicate that non-specific inhibitors like carbenoxolone can enhance cognitive function in humans and rodents. Inhibiting 11β-HSD1 may have neuroprotective effects by influencing metabolism and vascular health. 11β-HSD1 converts inactive cortisone into active cortisol, with its expression varying across fibroblasts in different tissues. Its expression and activity increase in response to inflammatory stimuli, especially in tissues involved in chronic inflammation [29].

The precise regulation of 11β-HSD1 enables modulation of inflammation, ensuring that the inflammatory response is appropriate and localized. These findings emphasize the critical role of 11β-HSD1 in regulating tissue during inflammatory processes, opening possibilities for targeted interventions to effectively control inflammation in localized areas [33][34].

1.4. Marine fungi compounds and their bioactive potential

1.4.1 Importance of marine fungi in the discovery of bioactive compounds

Marine fungi are an ecologically significant group that play crucial roles in marine environments. However, despite their importance, they are often overlooked in favor of other more extensively studied marine organisms, such as bacteria and algae. These fungi are highly diverse and can be found in virtually all marine habitats, from surface waters to deep ocean zones. They colonize a wide range of substrates, including sediments, corals, algae, and other marine organisms. The diversity among marine fungi is vast, with many species adapted to different environmental conditions. However, numerous organisms remain unclassified or undescribed, suggesting that actual diversity may be much greater than currently recognized. Marine fungi perform essential ecological functions in marine ecosystems. As decomposers, they break down organic matter and facilitate nutrient cycling, which is vital for the health and sustainability of these ecosystems. Additionally, many marine fungi form symbiotic relationships with organisms such as corals and algae, contributing to marine habitats' ecological dynamics and structure [30].

Marine fungi can inhabit or infect various organisms, including algae, corals, sponges, and other fungi. Additionally, primary producers, such as dinoflagellates and diatoms, are frequently affected by marine fungi. These interactions can significantly impact global carbon cycles. Although only a few studies have quantified the exact biomass of these fungi, evidence suggests that their biomass may even surpass that of bacteria, particularly in environments with high levels of organic carbon [31].

Research into natural products, especially marine fungi compounds, holds significant potential for exploration. Several studies have already highlighted the key advantages and disadvantages of the molecules extracted from these products, suggesting their potential as candidates for new drug development [31].

The marine environment provides a rich source of microorganisms, including bacteria, fungi, actinomycetes, cyanobacteria, and diatoms, which produce bioactive compounds [32]. These microbes generate substances that affect various systems in the body, such as the nervous, respiratory, cardiovascular and gastrointestinal systems. Among these organisms, marine fungi stand out, as they produce unique metabolites due to the extreme conditions of the ocean, such as temperature and salinity. Their compounds have the potential to develop new drugs, even in low concentrations, making them promising for pharmaceutical biotechnology [33].

Figure 7 illustrates the variety of bioactive compounds found in the marine environment. In 1949, Cephalosporin C was the first secondary metabolite isolated from a marine fungus near the coast of Sardinia. Although this discovery was significant, identifying new bioactive compounds from marine microorganisms has been a slow process. However, since the 1980s, research into the pharmacological potential of these compounds has increased. The ocean remains one of the least explored areas on the planet, particularly regarding the medicinal applications of microorganisms [34].

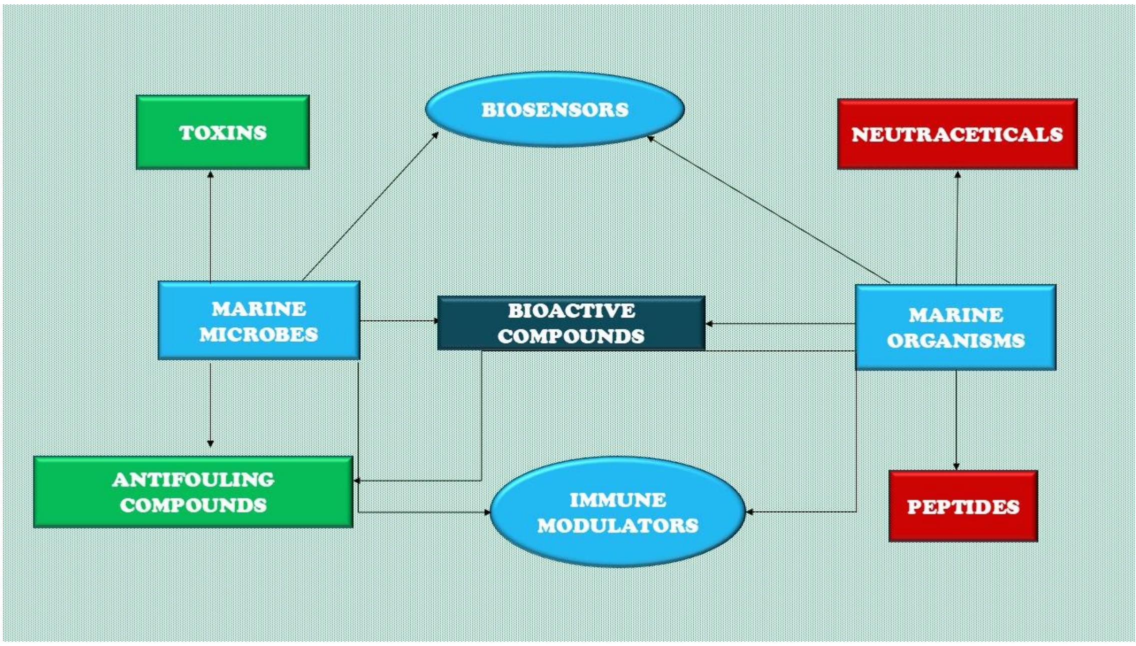


Figure 7- Bioactive compounds produced in the marine habitat [36].

1.4.2 Antibacterial and antifungal activities

Antibacterial and antifungal drugs are among the most widely used medications. However, the increasing resistance of bacterial and fungal pathogens has created a growing demand for new compounds with these properties. In this context, natural products derived from fungi are a promising source for developing new antibacterials and antifungals. This is due to the vast diversity of fungal species, the variety of their secondary metabolites, and advancements in genetic cultivation and fermentation techniques. Research has examined the antimicrobial activity of various fungi from different environments to identify new compounds, including endophytic fungi from wild plants and marine fungi. The genus *Aspergillus* stands out as one of the most significant genera of marine fungi, having produced more novel antibacterial and antifungal compounds than any other fungal genus [35].

1.4.3 Antiviral activity

The search for antiviral compounds derived from marine fungi has yielded promising results. In bioassay-based experiments, equisetin, fomasetin, and intrinsic acid have shown significant anti-HIV activities. For instance, sansalvamide A, a cyclic depsipeptide isolated from the marine fungus Fusarium sp., has demonstrated the ability to inhibit the topoisomerase of the pathogenic virus Molluscum contagiosum (MCV). It promotes this activity by binding to the DNA, thus blocking DNA relaxation catalyzed by the enzyme and forming covalent complexes (IC_{50} = 124 μM). The isolation and identification of this compound is particularly important because MCV can cause serious lesions in AIDS patients [36].

1.4.4 Anti-cancer activities

For several decades, marine invertebrates have been the focus of research for the discovery of potential anticancer compounds, serving as the primary sources for isolating these compounds [32]. In recent years, research has focused on discovering and isolating microorganisms associated with these species to evaluate their compounds and extracts for anticancer activity [37].

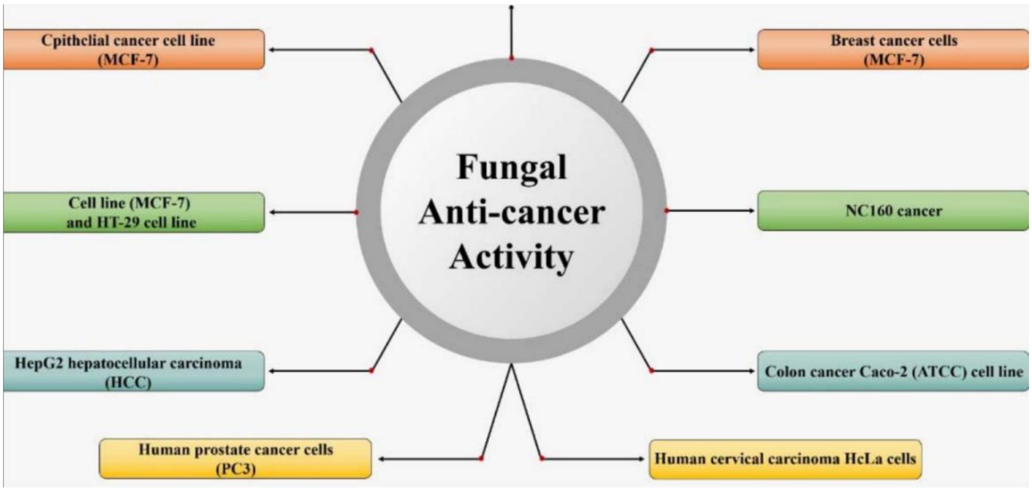


Figure 8- Anti-cancer activity of fungi in different cell-based models [40].

1.5 Bioinformatics

1.5.1 Bioinformatics for drug development

Bioinformatics is a field that integrates biology, computer science, statistics, and mathematics. It has driven significant advances in genomic sequencing, data processing, and the analysis of large amounts of complex data [38]. By connecting these disciplines, bioinformatics has made it possible to develop tools that increase feasibility, efficiency and effectiveness in the drug research and development process, using molecular modeling tools such as virtual screening and molecular docking, [39].

1.5.2 Virtual screening

Virtual screening involves using computational methods to analyze large compound databases and identify potential hit candidates in virtual libraries [40]. *In silico* experiments can complement high-throughput screening (HTS) and are often combined with screening campaigns. They can also be conducted before or after experimental screening to identify compounds that may not have been detected through *in vitro* readings, known as latent hits [41]. There are two main virtual screening approaches: ligand-based screening and receptor-based screening. In ligand-based screening, the 2D or 3D chemical structures or molecular descriptors of known active substances are used to find similar compounds in a database. In structure-based screening, the 3D structure of protein targets of interest is used to assess if a library of compounds is predicted to inhibit the protein target activity. This structure-based screening usually uses docking tools, molecular dynamic simulation tools and adequate scoring functions [47].

The molecule selection process is defined with a library of compounds so that the final candidate molecules are filtered to select the ones with the highest potential. In Figure 9, molecules with the potential to be good drug candidates are initially selected based on properties that favour their action or because they are similar to already known drugs. Next, the candidate ligands are analyzed, eliminating those whose pharmacophoric groups indicate a likelihood of toxicity. The best conformations for the candidate ligands are identified in the next step. During this process, the compositions and structures of the candidate ligands can be adjusted to improve their properties, especially their pharmacokinetic characteristics (absorption, distribution, metabolism, excretion and toxicity, ADMET). This process may require further cycles of optimization of the composition and structure of the candidate ligands. Thus, the ligands are prepared for biological testing after this process has been completed [41].

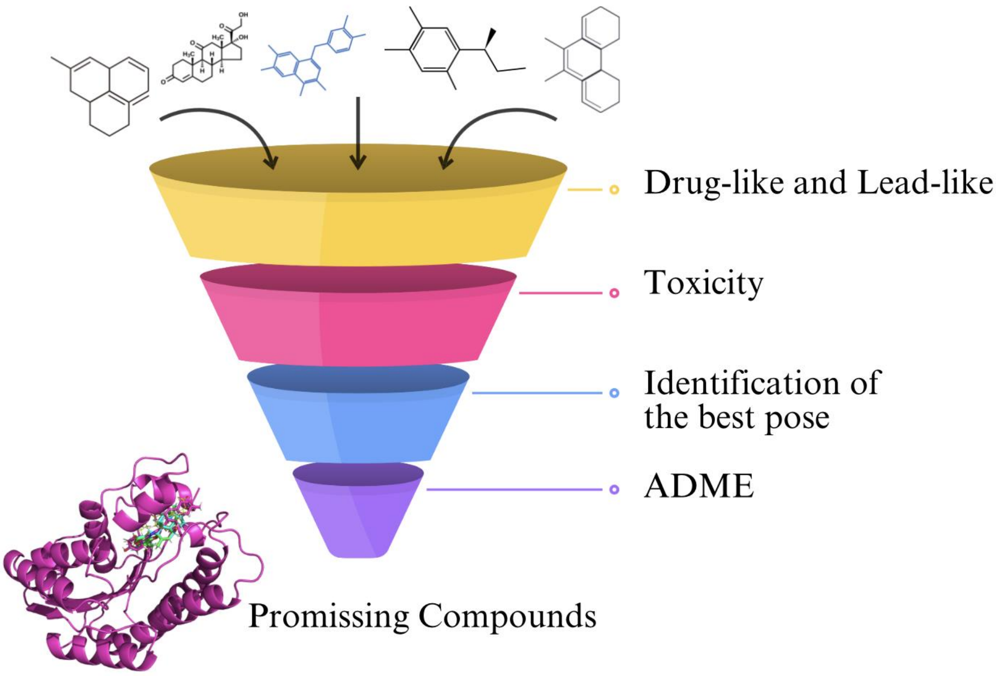


Figure 9- Virtual screening process. Self-authored (2024).

1.5.3 Molecular docking

In recent years, molecular docking has become essential in drug development. This method predicts how a small molecule may interact with a protein at the atomic level. It allows researchers to analyze the behavior of these molecules in the binding site of the target protein, thus gaining a better understanding of the biochemical processes involved. The technique is based on the structure of the protein, which requires a high-resolution three-dimensional representation obtained using X-ray crystallography (X-ray), nuclear magnetic resonance spectroscopy, or cryo-electron microscopy [42].

In recent decades, molecular docking has been used as an efficient and economical technique to predict how known active compounds bind to proteins. This approach is crucial for screening libraries of compounds, reducing costs and speeding up the process of discovering new drugs, as well as identifying promising compounds [43].

Molecular docking is a tool that predicts the interaction between a ligand (small molecule) and a receptor (usually a macromolecule). Depending on the type of molecule being studied, docking can be classified as follows [44]:

- Protein-Ligand: This type seeks to determine the best orientation and position of a ligand in relation to a target protein, predicting how the two molecules interact and what the best fitting geometry is [45].
- Protein-Protein: Focused on studying the potential interactions between different proteins [46].
- Protein-DNA: Examines how proteins interact with DNA, elucidating the mechanism of action of these interactions [46].

This technique has proven crucial in advancing drug discovery, offering detailed insights into molecular interactions and helping to optimize new therapeutic compounds. The type used in this work is protein-ligand docking, as shown in Figure 10.

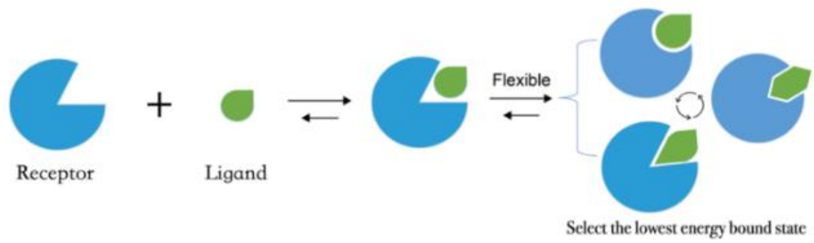


Figure 10- Induced fit model. Adapted from [49].

1.5.4 Molecular Docking Software (Protein-ligand): AutoDock VINA

Over the last twenty years, more than 60 docking tools and programs have been created for academic and commercial purposes. These include DOCK, AutoDock, FlexX, GOLD, ICM, Glide, Cdocker, LigandFit, MCDock, FRED, MOE-Dock, LeDock, AutoDock Vina, rDock, UCSF Dock and many others. In this work, was used AutoDock Vina (VINA) to perform the molecular docking processes with the support of the Yasara software to run the screening_dockrun.mcr script [48]. For example, the VINA docking algorithm generates a population of random spatial conformations for a compound expected to bind to a protein. Each conformation is evaluated using a scoring function that calculates the ΔGprev parameter, representing the predicted binding free energy. This function assesses the energy of the ligand and protein interaction. Therefore, a lower ΔGpred value indicates a more potent predicted inhibition of the compound in question [49].

2. Methodology

In this section we will describe in detail the computational approach used to study the inhibitory capacity of the library of bioactive compounds from marine fungi against the activity of the 11β-HSD1 protein in 3 important stages: the selection and preparation of the crystallographic structure of the target protein, the construction of a virtual library of compounds and the application of molecular docking and virtual screening tools.

2.1 Collection and preparation of X-ray structures of 11β-HSD1

To identify the best structures of the 11β-HSD1 protein, several steps were undertaken for editing and preparing the protein files (Figure 11). This process involved the following procedures:

1. Obtaining the Protein: The Protein Data Bank (https://www.rcsb.org/) provided information and '.pdb' files for analysis.

2. Structure Analysis: To select the most suitable structure, it needed to meet the following criteria: it had to be from *Homo sapiens*, present no mutations, be obtained through X-ray crystallography, have a resolution below 2,5 Å, include a co-crystallized ligand, and possess an experimental inhibition value for this ligand. In the Protein Data Bank (PDB), only one structure met these requirements.

3. Editing the '.pdb' File: After selecting the most suitable X-ray structure of 11β-HSD1, the '.pdb' file was manually using notepad. This involved removing the HETATM lines corresponding to the ligands and co-crystallized molecules and deleting the TER, CONNECT, and HETATM lines.

4. Preparation in YASARA: The YASARA software has adjusted the protein in '.pdb'. format. Several modifications were made to the structure during this process: (1) Gasteiger charges were added, and (2) hydrogen atoms were included.

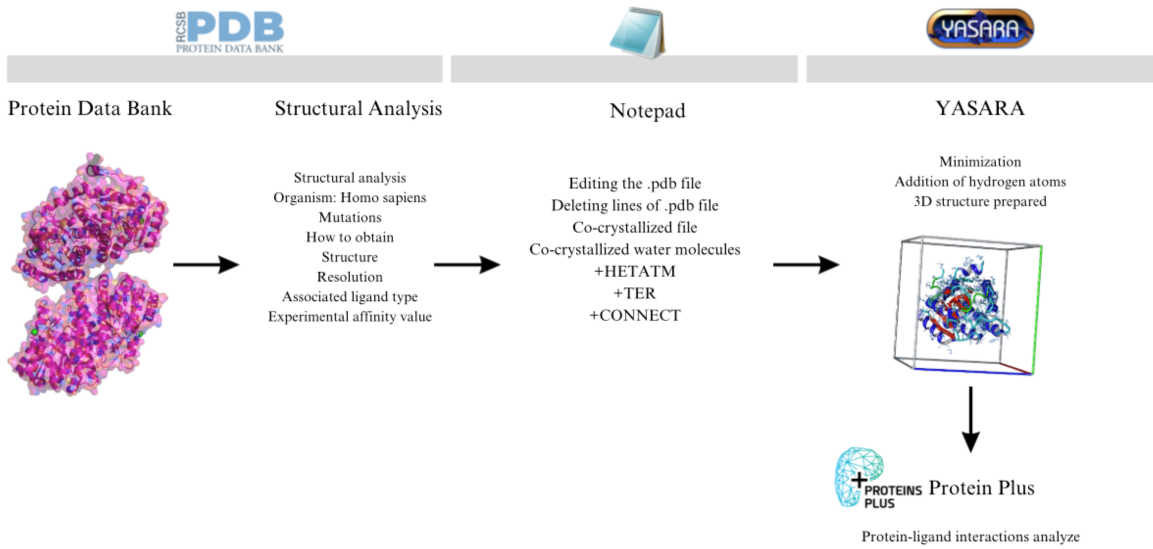


Figure 11- Protein preparation scheme. Self-authored (2024).

2.2 Preparing a library of marine fungi compounds

Based on a review conducted by Deshmukh *et al*. (2018) and Calhelha *et al*. (2023), a virtual library of 157 compounds isolated from marine fungi was created to compare their efficacy to a library of commercial compounds previously studied and tested by Cabrera Pérez, L. C. *et al.* (2020). After identifying each compound, we started a virtual process involving designing, preparing, and optimizing three-dimensional (3D) molecular structures. Initially, each compound was drawn in two-dimensional (2D) format before being converted into 3D format. Initially, the compounds of marine origin were selected in SMILES format corresponding to each compound that was obtained. This step was carried out using a literature search. The structures were optimized in the YASARA software [55] for virtual screening.

2.3. Docking and virtual screening procedures

The files were processed using the dock_runscreening.mcr script (Figure 12), an automated tool designed for large-scale docking screenings using VINA software. This script organizes and standardizes the steps involved in docking simulations, enabling the evaluation of interactions between small molecules (ligands) and the protein target.

```
dock_runscreening.mcr
# YASARA MACRO
# TOPIC:       5. Structure prediction
# TITLE:       Docking many ligands to a receptor to perform virtual screening
# REQUIRES:    Structure
# AUTHOR:      Elmar Krieger
# LICENSE:     GPL
# DESCRIPTION: This macro runs VINA or AutoDock to dock multiple ligands against a receptor and saves a sorted table of
their binding energies, as well as the corresponding complexes

# Parameter section - adjust as needed, but NOTE that some changes only take effect
# if you start an entirely new docking job, not if you continue an existing one.
# ============================================================================

# You can either set the target structure by clicking on Options > Macro > Set target,
# by providing it as command line argument (see docs at Essentials > The command line),
# or by uncommenting the line below and specifying it directly.
#MacroTarget '/home/myname/projects/docking/1sdf'

# Docking method, either AutoDockLGA or VINA
method='VINA'

# Number of docking runs per ligand (maximally 999, 0 lets YASARA choose based on the number of CPUs)
runs=0

# Number of best poses per ligand saved and reported
bestposes=1

# Set to 1 to keep the ligand completely rigid (alternatively you can provide
# the ligand as a *.yob file and fix certain dihedral angles only).
rigid=0

# A selection of receptor residues whose side-chains should be kept flexible, e.g.
# flexres='Lys 91 Leu 100', or (if the receptor is not a monomer)
# flexres='Res Lys 91 Mol A or Res Leu 100 Mol B'.
# Alternatively you can provide the receptor as a *.sce or *.yob file with fixed
# atoms, which gives better control (e.g. you can keep only part of a side-chain
# or even a terminal backbone flexible).
flexres=''

# Sort results either by ligand number ('ligandnum'), binding energy ('bindnrg') or by
# efficiency ('effi'), i.e. binding energy per heavy atom, which addresses the bias towards
# larger ligands in screening.
sortby='bindnrg'
```

Figure 12- Script representation dock_runscreening.mcr, run on YASARA software.

Several critical parameters are fine-tuned during this process, including the search space (grid) configuration and the definition of interactions of interest. After executing Dock_runscreening.mcr, the results of the simulations were analyzed using Protein Plus software [56]. This platform specializes in visualizing and analyzing protein structures and molecular interactions, making it essential for interpreting the generated data. It allows the three-dimensional visualization of protein-ligand complexes, identification of potential binding interactions (such as hydrogen bonds, hydrophobic interactions, and ionic bonds), and quantitative analysis of binding affinity scores.

2.3.1 Redocking (validation of simulation)

After docking, it is necessary to conduct a validation process known as re-docking. This involves docking the co-crystallized inhibitor, Carbenoxolone (CBO) to the target protein (11β-HSD1) to verify that the software and parameters used can accurately reproduce the experimental pose of CBO.

2.3.2 Calculation of predicted inhibition (Ki_{pred})

The experimental inhibition constant data ($_{Kiexd}$) was obtained from the PDB database using the 2BEL structure with the CBO inhibitor and the predicted inhibition constant data ($_{Kipred}$) was obtained from simulations using the VINA software, which exports the free energy result (ΔG), which needs to be converted to Ki using the formula:

$$Kipred = \exp\left(\frac{\Delta Gpred * 1000}{Rcal * TK}\right)$$

where:

ΔG_{pred} is the predicted binding free energy calculated by VINA, Rcal is 1,98719 cal · K^{-1} · mol^{-1} e Temperature is 298,15 K. After obtaining the Ki_{pred} data, it was compared with the data from the PDB.

3. Results and Discussion

This research explores the potential of Marine Fungi compounds in treating type 2 diabetes. Many bioactive compounds derived from marine fungi possess pharmacological relevance, including possible applications for metabolic diseases. The establishment of this virtual Marine Fungi (MF) library serves as a valuable foundation for future research to develop new treatments for type 2 diabetes (in this study, as potential 11β-HSD1 inhibitors) and for treating other diseases.

3.1 11β-HSD1 X-ray selection and Re-Docking analysis

To select the most appropriate experimental X-ray structure of the protein target of interest, in this case, human 11β-HSD1, a search was performed in the Protein Data Bank (PDB) database. Eight PDB structures were found, and the main characteristics of each structure are presented in Table 2. All structures were experimentally obtained using the X-ray crystallography method, showing a resolution below 3,5 Å. In general, the lower the resolution value, the more accurate the protein structure is, and any value below the 2.5 Å threshold indicates that the X-ray structure is of good quality. For this reason, only structures with resolution values below 2.5 Å were considered for selection.

Also, the presence of co-crystallized ligands or known inhibitors was considered when selecting the structure. In general, it is important that the X-ray structure presents a compound bound to the protein structure, as this will enable the possibility of performing a Re-Docking analysis of the inhibitor. This means that molecular docking of the ligand can be performed, and the predicted binding conformation can be compared with the experimental binding conformation.

For the X-ray structures that present a co-crystallized binding inhibitor, a value of binding inhibition is sometimes available, usually an experimental constant of inhibition (Ki). This value being available can be important because the Re-Docking analysis also presents a predicted Ki value. This predicted Ki value can then be compared to the experimental Ki value, and the difference between predicted and experimental Ki is expected to be the lowest possible.

Considering all those factors, only one structure fitted all the parameters, PDB: 2BEL (Table 2). This 2BEL structure presented a resolution value of 2.11 Å (below the 2,5 Å considered threshold), presented a ligand (Carbenoxolone) and was the only X-ray structure where the experimental Ki value of the ligand was known. In this case, Carbenoxolone, a known 11β-HSD1 inhibitor with a Ki value of 61 nM.

Table 2- X-ray Structures of the 11β-HSD1. Self-authored (2024).

PDB	METHOD	RESOLUTION	LIGAND	Ki (nM)
1XU7	X-ray	1.80 Å	No	-
1XU9	X-ray	1.55 Å	No	-
2BEL	X-ray	2.11 Å	Carbenoxolone	62
2ILT	X-ray	2.30 Å	Adamantane Sulfone	No
2IRW	X-ray	3.10 Å	Adamantane Ether	No
2RBE	X-ray	1.90 Å	2-anilinothiazolone	No
3BYZ	X-ray	2.69 Å	2-Amino-1,3-thiazol-4(5H)-one	No

With the X-ray structure selected, the Re-docking analysis was performed. The 2BEL structure was prepared according to the protocol in Methodology and used as the protein target, and the Carbenoxolone structure was also adequately prepared. The docking study was performed, and the predicted binding conformation is present in Figure 13. As can be observed, the predicted and experimental structures closely align. This indicates that the VINA docking algorithm performs well when using this 2BEL structure for this type of docking analysis. This re-docking analysis is also sometimes called a positive control analysis, as we verify whether a known inhibitor, in this case, Carbenoxolone, is predicted by VINA to be an inhibitor.

Vina software also provides a predicted binding energy (ΔG) value. The lower this value is, the more potent the predicted inhibition is. For Carbenoxolone, the predicted ΔG value obtained was -10.8 Kcal/mol. According to the equation presented in the methodology, this value can also be translated into a predicted Ki value. In this case, the Carbenoxolone value of -10.8 Kcal/mol translates into a predicted Ki value of 12 nM. This predicted value is in the same order of magnitude as the known experimental Ki value of 60 nM for Carbenoxolone. The docking algorithms available, specifically VINA, have limitations in their prediction power, as they depend on protein structure quality. Also, the docking studies use a rigid protein structure that doesn't consider protein movement. So, obtaining predicted Ki (12 nM) and experimental Ki (60 nM) values in the same order of magnitude is a good indication that the selected X-ray structure and VINA software are a good combination to be used to predict the inhibition potential of other compounds. The compounds that will be subsequently analyzed are those present in the natural MF library, also prepared in this study.

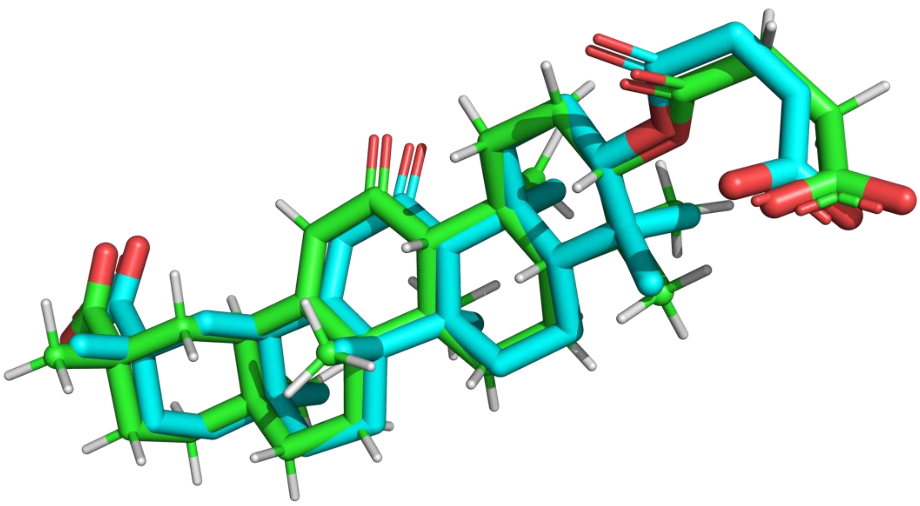
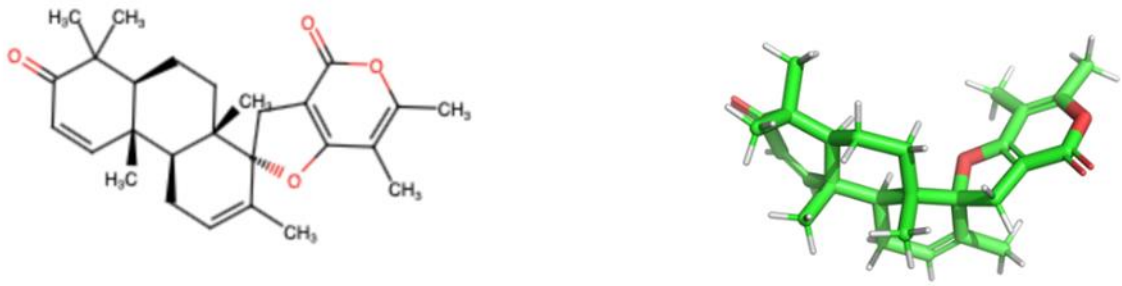


Figure 13- Alignment of the experimental binding conformation (light blue) and the predicted Carbenoxolone binding conformation at the 11β-HSD1 catalytic site in (green), both in stick format

3.2 Marine Fungi (MF) library construction

After selecting the most suitable PDB structure of human 11β-HSD1, we selected the compounds found in marine fungi species that presented some type of bioactivity. The compound information was collected from [57] and [58] reviews, which outlined the current knowledge of known marine fungi activities. All 157 compound virtual structures were prepared in adequate file formats for subsequent molecular modeling studies. The complete MF library is represented in 2D in Figures 15, 16 and 17. In general, it can be observed that there is a large diversity of types and complexity of compounds represented in the library. This MF library is an initial preparation effort with relatively few compounds. The number of compounds in this library is still limited by the number of marine fungi species studied and discovered. It is expected that going forward, the number of compounds to be added to the library will increase as more marine fungi species are discovered and adequately characterized. An example of one of the compounds present in the MF library is Brevine A, isolated from the deep-sea fungus *Penicillium* sp., which has a cytotoxic bioactivity against MCF-7 cell lines (Figure 14). In the future, this library will be made available for other researchers interested in using it for virtual screening studies with other molecular modeling tools and/or studying other protein targets of interest.



(A) (B)

Figure 14- 2D to 3D structure representation of the Brevinone A compound in the marine fungi library. Self-authored (2024).

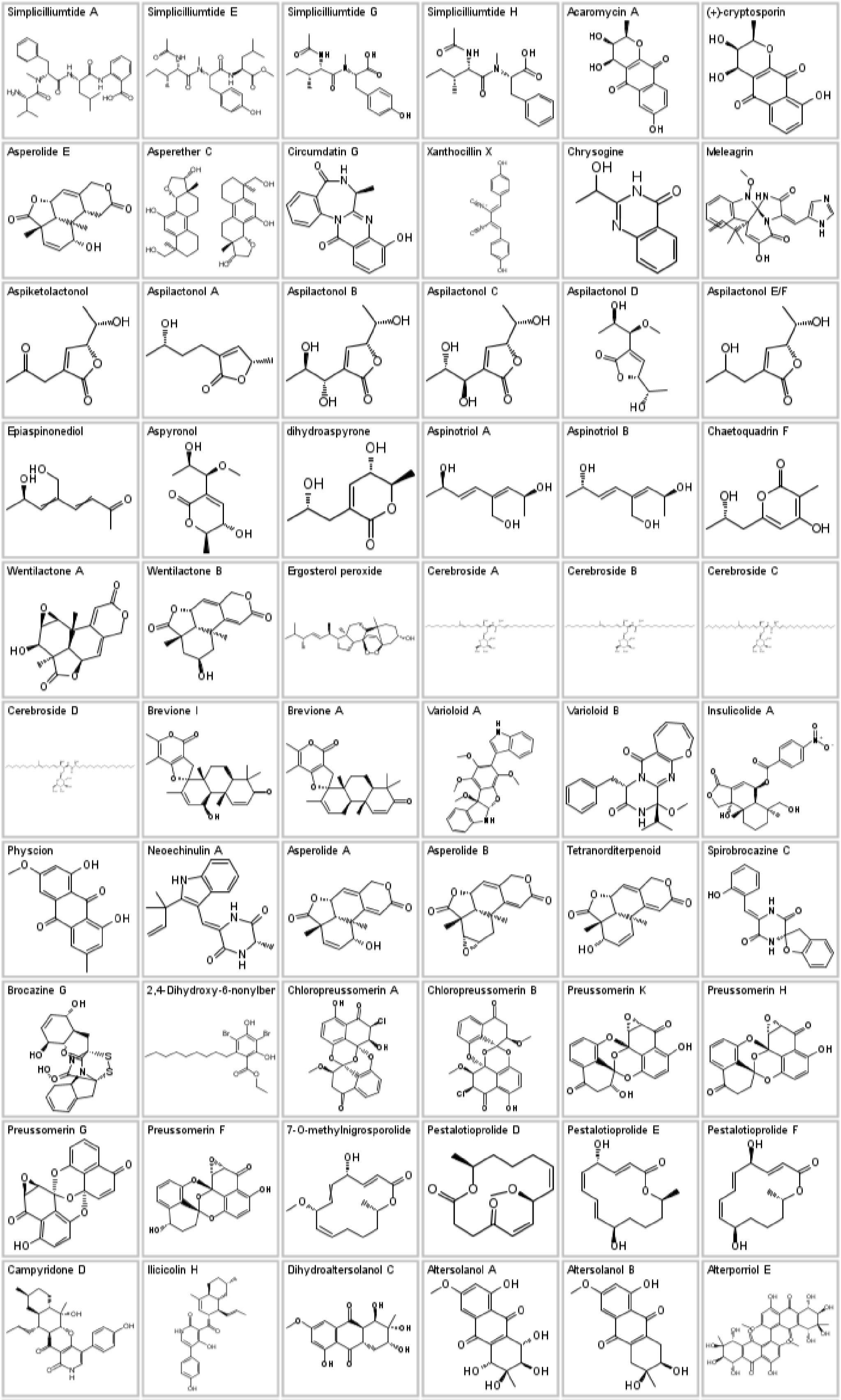


Figure 15- Representation of the compounds (1-60) in the marine fungi library. Self-authored (2024).

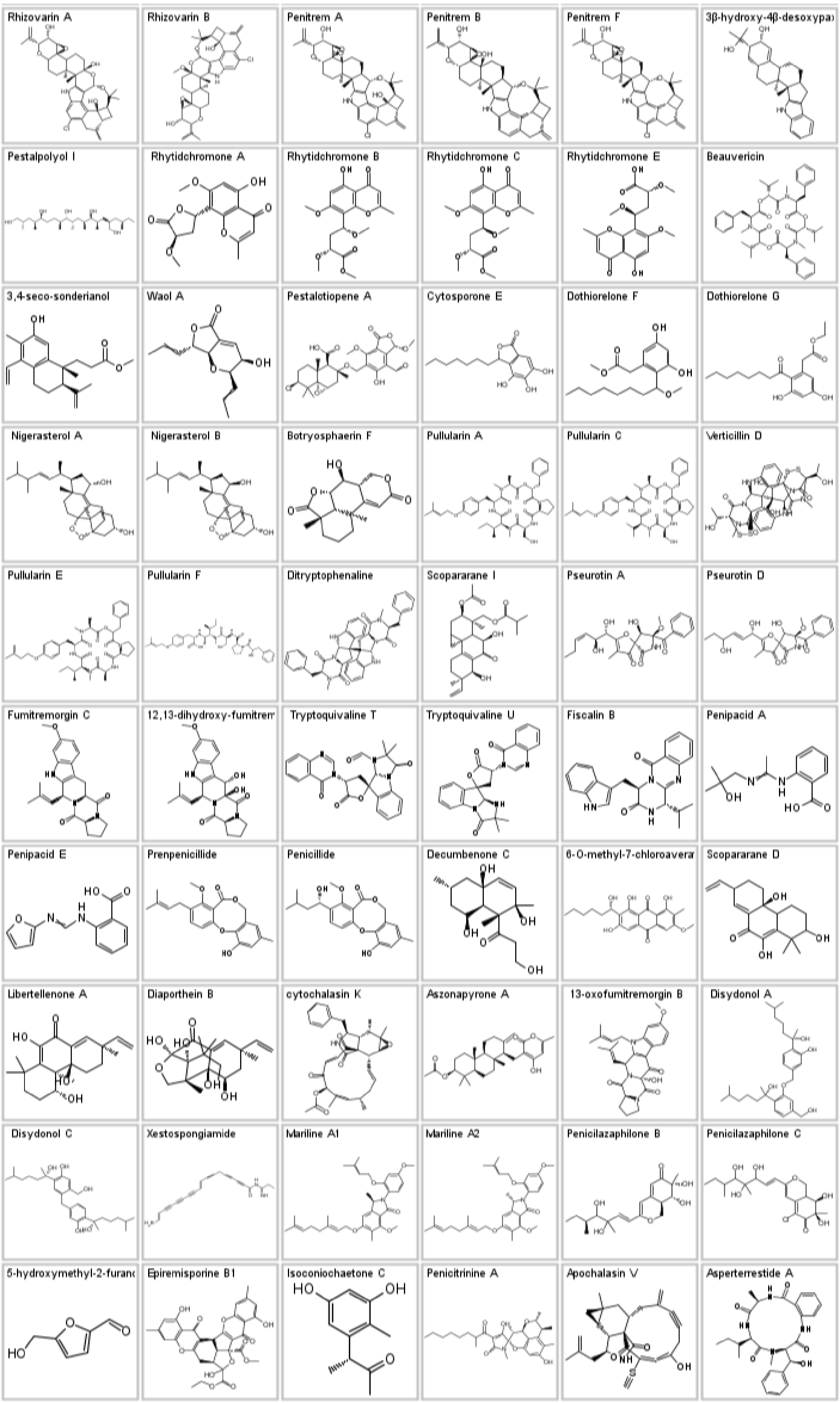

Rhizovarin A
Rhizovarin B
Penitrem A
Penitrem B
Penitrem F
3β-hydroxy-4β-desoxypa
Pestalpolyol I
Rhytidchromone A
Rhytidchromone B
Rhytidchromone C
Rhytidchromone E
Beauvericin
3,4-seco-sonderianol
Waol A
Pestalotiopene A
Cytosporone E
Dothiorelone F
Dothiorelone G
Nigerasterol A
Nigerasterol B
Botryosphaerin F
Pullularin A
Pullularin C
Verticillin D
Pullularin E
Pullularin F
Ditryptophenaline
Scopararane I
Pseurotin A
Pseurotin D
Fumitremorgin C
12,13-dihydroxy-fumitrem
Tryptoquivaline T
Tryptoquivaline U
Fiscalin B
Penipacid A
Penipacid E
Prenpenicillide
Penicillide
Decumbenone C
6-O-methyl-7-chloroavera
Scopararane D
Libertellenone A
Diaporthein B
cytochalasin K
Aszonapyrone A
13-oxofumitremorgin B
Disydonol A
Disydonol C
Xestospongiamide
Mariline A1
Mariline A2
Penicilazaphilone B
Penicilazaphilone C
5-hydroxymethyl-2-furanc
Epiremisporine B1
Isoconiochaetone C
Penicitrinine A
Apochalasin V
Asperterrestide A

Figure 16- Representation of the compounds (60-120) in marine fungi library. Self-authored (2024)

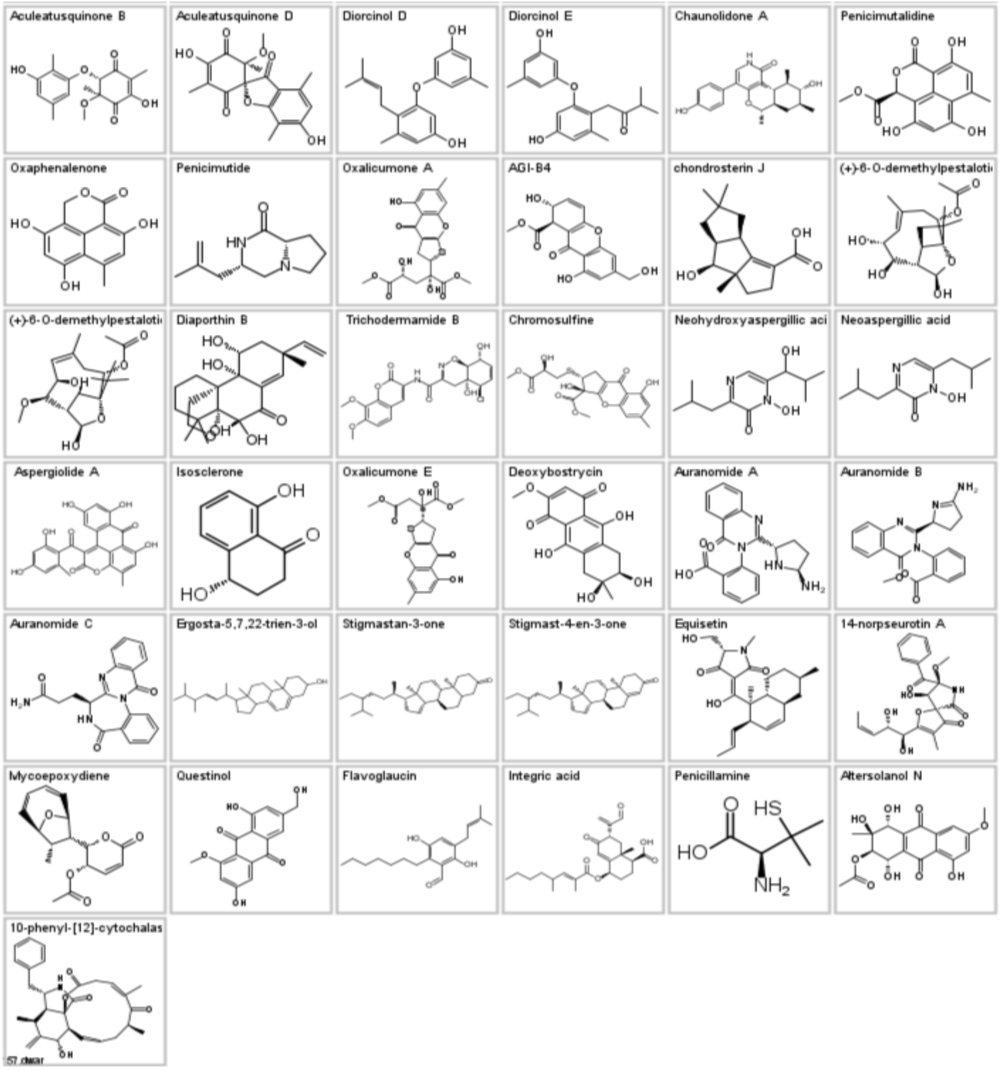


Figure 17- Representation of the compounds (120-157) in marine fungi library. Self-authored (2024).

3.3. Virtual Screening Results of the MF library against 11β-HSD1

Table 3 lists the 3 best compounds predicted to inhibit the 11β-HSD1 enzyme, their binding energies, and the receptor residues they are predicted to interact with. Among the compounds evaluated from the MF library: Brevione A (33), Brevione I (32), Ilicicolin H (56), and the reference inhibitor Carbenoxolone are presented in Figure 18. Brevione A (33) is the most efficient inhibitor, exhibiting the most negative binding energy of -11.6 kcal/mol, which indicates a high affinity for the enzyme's active site. Brevione I (32) follows closely, with a binding energy of -11.35 kcal/mol, suggesting it has similar efficacy, albeit slightly lower. Ilicicolin H (56) has a binding energy of -10.9 kcal/mol, indicating a slightly lower affinity than Brevione A and Brevione I. The reference inhibitor Carbenoxolone, which is co-crystallized with the enzyme, has a higher binding energy of -10.8 kcal/mol, providing a benchmark for evaluating the new compounds, which display greater affinity (Figure 18).

The low value predicted binding energies indicate these three compounds may be considered potential inhibitors of 11β-HSD1, although these predictions must be experimentally validated.

Regarding the contact residues in the active site of 11β-HSD1, Brevione A (33) and Brevione I (32) interact with a similar set of residues, including ILE 46, and a series of other residues (ASN 119 through VAL 227). This similarity suggests that both compounds occupy the active site in a comparable manner, forming critical interactions that stabilize the protein-ligand complex. In contrast, Ilicicolin H (56) interacts with additional residues, including GLY 45, GLY 47, and others (PRO 178 through ILE 230), which may account for its slightly lower affinity compared to the Brevione compounds. Although Ilicicolin H has a different interaction network, it still binds effectively to the active site of 11β-HSD1, albeit with reduced affinity. Carbenoxolone, the co-crystallized inhibitor, interacts with almost all the same residues as the other compounds, demonstrating consistency in its interactions within the enzyme's active site. Its binding energy of -10.8 kcal/mol serves as an established standard for efficacy, indicating that the new compounds, particularly Brevione A (33) and Brevione I (32), have significant potential to be even more effective inhibitors of 11β-HSD1.

Table 3- Binding Affinity and Contact Residues of Compounds with 11β-HSD1. Self-authored (2024).

Compound	**Predicted Binding energy [kcal/mol]**	**Contacting 11β-HSD1 residues**
Brevione A (33)	-11.6	Tyr183A, Thr220A and Ala223A
Brevione I (32)	-11.4	Tyr183A, Ser170A, Asn119A, Val180A and Leu126A
Ilicicolin H (56)	-10.9	Tyr183A, Thr220A, Ala223A and Val227A.
Carbenoxolone (Co-cristal inhibitor) (12)	-10.8	Tyr183A, Ser170A, Met233A and Leu217A.

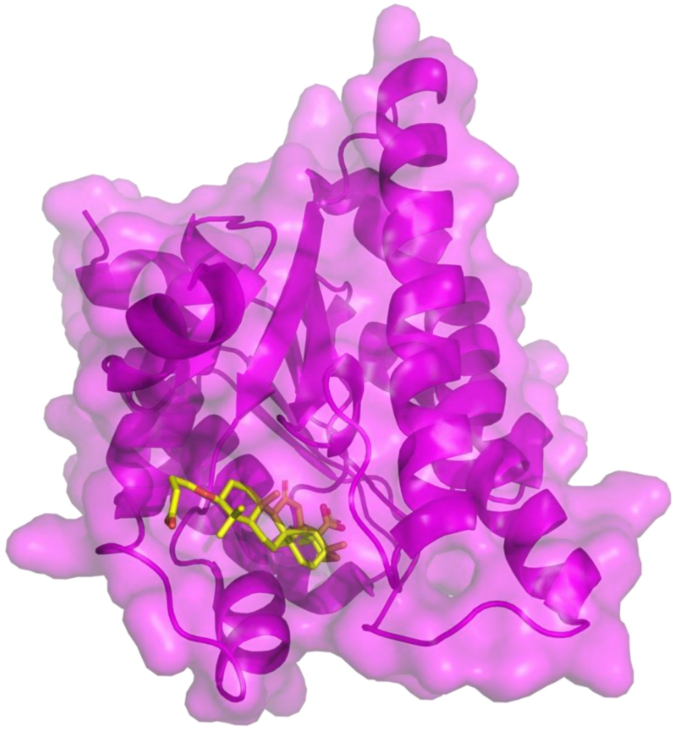


Figure 18- Representation of Structure of human 11β-HSD1 in complex with carbenoxolone (PDB:2BEL)

3.4. Detailed analysis of the predicted binding conformation of the most promising compounds against 11β-HSD1

The binding conformation of the three most promising MF library compounds against 11β-HSD1 is depicted in Figures 19 and 21. Figure 19 illustrates molecular complex representations of the protein-ligand interactions. Figures 20 and 21 represent the interaction diagrams that detail the specific interactions between the protein and the ligands. These diagrams effectively visualize hydrogen bonds, hydrophobic interactions, polar interactions, and other intermolecular forces that stabilize the protein-ligand complex. Such interactions, particularly hydrogen bonds formed with enzyme residues, are crucial for the efficacy of the compounds as 11β-HSD1 inhibitors. Analyzing the different conformations and interactions of the ligands provides valuable insights into identifying promising candidates for inhibiting the enzyme, which will require future experimental validation.

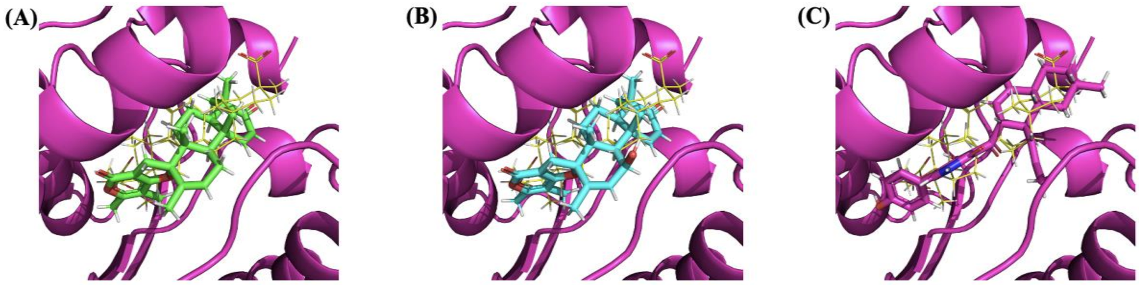


Figure 19- The potential inhibitory compounds were represented in stick format: (A) Brevione A and represented in green, (B) Brevione I represented in light blue and (C) Ilicicolin H in pink. The Control Carbenoxolone is represented in yellow in line format. Self-authored (2024).

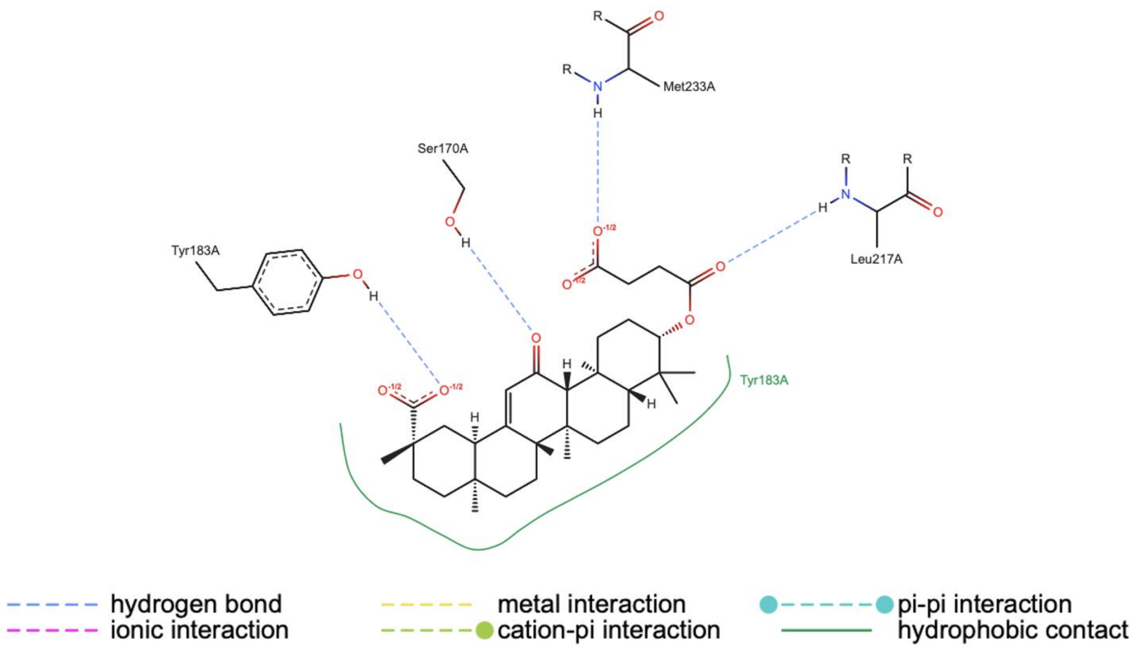


Figure 20- Interactions between the Carbenoxolone and the receptor's residues

Figure 21 shows CBO interacting with residues of the 11β-HSD1 protein through hydrogen bonds and hydrophobic interactions. The residues that constitute the hydrogen bonds with CBO are: Tyr183A, Ser170A, Met233A and Leu217A. The residues Tyr183A and Ser170A form hydrogen bonds with CBO with their hydroxyl groups, which stabilizes CBO in the binding site. On the other hand, residues Met233A and Leu217A form hydrophobic interactions with nearby oxygen atoms, helping to fix the molecule. These structural interactions - hydrogen bonds and hydrophobic contact - are fundamental for CBO to position itself and stabilize in the protein's binding site, allowing it to act as a modulator of anti-inflammatory and anti-diabetic effects. This detailed understanding of interactions could also be useful in guiding the development of CBO analogues with greater potency and selectivity.

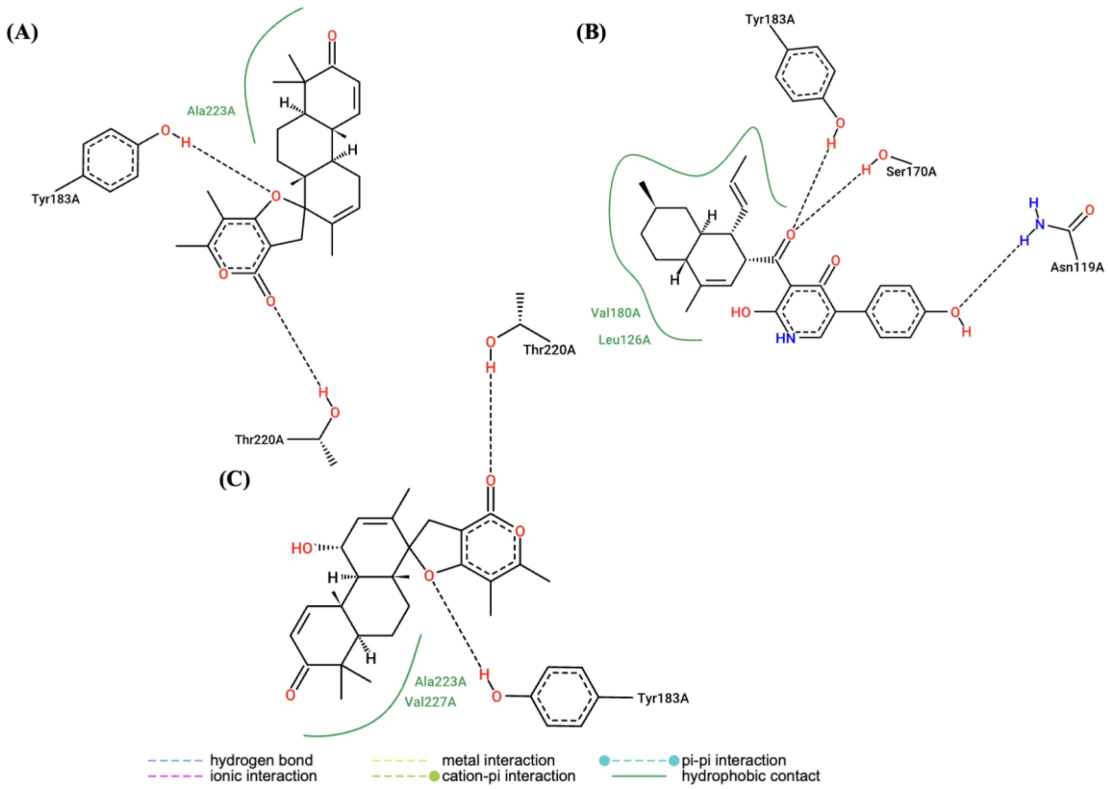


Figure 21- Representation of the molecular interactions between the receptor and the compounds (A) Brevione A, (B) Brevione I present in *Penicillium* sp. and (C) Ilicicolin H, present in *Campylocarpon* sp. HDN13-307 marine fungi. Self-authored (2024).

Out of the 157 compounds present in the MF library, three compounds emerged with notable predicted affinities: Brevione A, Brevione I and Ilicicolin, with Kipred values of

3.14 nM, 4.80 nM, and 10.25 nM, respectively, compared to CBO, which has an experimental Ki_{exp} of 60 nM.

The Ser170 residue interacted with the control (CBO) and was also found to exhibit interactions with other known 11β-HSD1 inhibitors, including BPH [23], LG13 [24], and the 1,2,4-triazole derivatives [25]. The compound Brevione I (32) also showed predicted hydrogen bonding interactions with Ser170 residue. For Tyr183 residue, hydrogen bonding interactions were also observed with CBO, BPH [23], and the 1,2,4-triazole derivatives [25], as well as were predicted to interact with Brevione A (33), Brevione I (32), and Ilicicolin H (56). The Thr220 residue did not interact with CBO (the control) but was predicted to form a hydrogen bond with Brevione A (33) and Ilicicolin H (56). The Asn119A residue was predicted to interact solely with Brevione I (32). In contrast, the Ala223 residue was predicted to form hydrophobic interactions with Brevione A (33), Ilicicolin H (56) in a similar fashion to 1,2,4-triazole derivatives [25]. Val180A residue was predicted to form hydrophobic interactions with Brevione I (32) and the 1,2,4-triazole derivatives [25]. Additionally, the Leu126A residue was predicted to form hydrophobic interactions with Brevione I (32), like the known inhibitors LG13 [25] and 1,2,4-triazole derivatives [25], while the Val227A residue was predicted to interact with Ilicicolin H and LG13 [24].

4. Conclusion

11β-HSD1 is an enzyme that converts cortisone into cortisol, a hormone that regulates inflammatory processes, glucose metabolism, and fat storage. Overexpression of 11β-HSD1 is linked to type 2 diabetes, obesity, and chronic inflammatory conditions. Therefore, developing inhibitors for 11β-HSD1 could be a promising strategy for treating these conditions by lowering cortisol levels in affected tissues and alleviating harmful inflammatory and metabolic responses. A virtual Marine Fungi (MF) library of 157 molecules derived from marine fungi was created. The selected compounds presented some bioactive potential, particularly anti-cancer, antibacterial, and antiviral activities. A virtual screening of the MF library was performed using VINA and YASARA softwares. Among the compounds evaluated, three demonstrated superior predicted 11β-HSD1 inhibition activity compared to the control (carbenoxolone). Specifically, Brevione A (33), Brevione I (32), and Ilicicolin H (56), that are present in Penicillium *sp.* and Campylocarpon sp. HDN13-307 exhibited significant predicted binding energy values of -11.6 kcal/mol, -11.34 kcal/mol, and -10.9 kcal/mol, respectively, translated into the predicted constant of inhibition (Ki_{prev}) of 3.14 nM, 4,80 nM and 10.25 nM, comparing well with carbenoxolone, that has a Ki_{exp} of 60 nM. A detailed analysis of the predicted binding conformation was performed, and the main residues of the catalytic site involved in the interaction were discussed. These results indicate a high potential of the three compounds as inhibitors of 11β-HSD1, highlighting the need for experimental validation to confirm their efficacy and mechanisms of action.

5. References

[1] N. G. Forouhi and N. J. Wareham, "Epidemiology of diabetes," *Medicine*, vol. 38, no. 11, pp. 602–606, Nov. 2010, doi: 10.1016/j.mpmed.2010.08.007.

[2] H. Yaribeygi, F. R. Farrokhi, A. E. Butler, and A. Sahebkar, "Insulin resistance: Review of the underlying molecular mechanisms," *J Cell Physiol*, vol. 234, no. 6, pp. 8152–8161, Jun. 2019, doi: 10.1002/jcp.27603.

[3] R. Modzelewski, M. M. Stefanowicz-Rutkowska, W. Matuszewski, and E. M. Bandurska-Stankiewicz, "Gestational Diabetes Mellitus—Recent Literature Review," *J Clin Med*, vol. 11, no. 19, p. 5736, Oct. 2022, doi: 10.3390/JCM11195736.

[4] I. Vlad and A. R. Popa, "Epidemiology of Diabetes Mellitus: A Current Review," *Rom J Diabetes Nutr Metab Dis*, vol. 19, no. 4, pp. 433–440, Dec. 2012, doi: 10.2478/v10255-012-0050-0.

[5] D. Glovaci, W. Fan, and N. D. Wong, "Epidemiology of Diabetes Mellitus and Cardiovascular Disease," *Curr Cardiol Rep*, vol. 21, no. 4, pp. 1–8, Apr. 2019, doi: 10.1007/S11886-019-1107-Y/FIGURES/3.

[6] G. Wilcox, "Insulin and insulin resistance.," *Clin Biochem Rev*, vol. 26, no. 2, pp. 19–39, May 2005.

[7] S. Schinner, W. A. Scherbaum, S. R. Bornstein, and A. Barthel, "Molecular mechanisms of insulin resistance," *Diabetic Medicine*, vol. 22, no. 6, pp. 674–682, Jun. 2005, doi: 10.1111/j.1464-5491.2005.01566.x.

[8] G. Sesti, "Pathophysiology of insulin resistance," *Best Pract Res Clin Endocrinol Metab*, vol. 20, no. 4, pp. 665–679, Dec. 2006, doi: 10.1016/j.beem.2006.09.007.

[9] “Mechanism of action of insulin | BioRender Science Templates.” Accessed: Oct. 24, 2024. [Online]. Available: https://www.biorender.com/template/mechanism-of-action-of-insulin

[10] S. R. Colberg *et al.*, “Exercise and Type 2 Diabetes,” *Diabetes Care*, vol. 33, no. 12, pp. e147–e167, Dec. 2010, doi: 10.2337/dc10-9990.

[11] “The Diabetes Prevention Program (DPP),” *Diabetes Care*, vol. 25, no. 12, pp. 2165–2171, Dec. 2002, doi: 10.2337/diacare.25.12.2165.

[12] T. R. Gray A, *Nutritional Recommendations for Individuals with Diabetes*. 2000.

[13] A. B. Evert *et al.*, “Nutrition Therapy Recommendations for the Management of Adults With Diabetes,” *Diabetes Care*, vol. 37, no. Supplement_1, pp. S120–S143, Jan. 2014, doi: 10.2337/dc14-S120.

[14] “Mediterranean Nutrition | Active CreteActive Crete.” Accessed: Oct. 29, 2024. [Online]. Available: https://activecrete.gr/mediterranean-nutrition/

[15] A. Chaudhury *et al.*, “Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management,” *Front Endocrinol (Lausanne)*, vol. 8, Jan. 2017, doi: 10.3389/fendo.2017.00006.

[16] K. Kupai *et al.*, “Recent Progress in the Diagnosis and Management of Type 2 Diabetes Mellitus in the Era of COVID-19 and Single Cell Multi-Omics Technologies,” *Life*, vol. 12, no. 8, p. 1205, Aug. 2022, doi: 10.3390/life12081205.

[17] R. Patel, J. Williams-Dautovich, and C. L. Cummins, “Minireview: New Molecular Mediators of Glucocorticoid Receptor Activity in Metabolic Tissues,” *Molecular Endocrinology*, vol. 28, no. 7, pp. 999–1011, Jul. 2014, doi: 10.1210/ME.2014-1062.

[18] S. Dodd, D. R. Skvarc, O. M. Dean, A. Anderson, M. Kotowicz, and M. Berk, “Effect of Glucocorticoid and 11β-Hydroxysteroid-Dehydrogenase Type 1 (11β-HSD1) in Neurological and Psychiatric Disorders”, doi: 10.1093/ijnp/pyac014.

[19] I. J. Bujalska, S. Kumar, and P. M Stewart, "Does central obesity reflect 'Cushing's disease of the omentum'?," *The Lancet*, vol. 349, no. 9060, pp. 1210–1213, Apr. 1997, doi: 10.1016/S0140-6736(96)11222-8.

[20] A. E. Michael, L. M. Thurston, and M. T. Rae, "Glucocorticoid metabolism and reproduction: a tale of two enzymes," *Reproduction*, vol. 126, no. 4, pp. 425–441, Oct. 2003, doi: 10.1530/REP.0.1260425.

[21] P. M. Jamieson, K. E. Chapman, C. R. Edwards, and J. R. Seckl, "11 beta-hydroxysteroid dehydrogenase is an exclusive 11 beta- reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations," *Endocrinology*, vol. 136, no. 11, pp. 4754–4761, Nov. 1995, doi: 10.1210/ENDO.136.11.7588203.

[22] R. Shukla *et al.*, "11β Hydroxysteroid dehydrogenase - 1 activity in type 2 diabetes mellitus: A comparative study," *BMC Endocr Disord*, vol. 19, no. 1, pp. 1–9, Jan. 2019, doi: 10.1186/S12902-019-0344-9/TABLES/6.

[23] H. Wang *et al.*, "Bisphenol A Analogues Inhibit Human and Rat 11β-Hydroxysteroid Dehydrogenase 1 Depending on Its Lipophilicity," *Molecules*, vol. 28, no. 13, p. 4894, Jul. 2023, doi: 10.3390/MOLECULES28134894/S1.

[24] L. Zhao *et al.*, "Inhibition of 11β-HSD1 by LG13 improves glucose metabolism in type 2 diabetic mice," *J Mol Endocrinol*, vol. 55, no. 2, pp. 119–131, Oct. 2015, doi: 10.1530/JME-14-0268.

[25] D. A. Osman *et al.*, "Structural Insights and Docking Analysis of Adamantane-Linked 1,2,4-Triazole Derivatives as Potential 11β-HSD1 Inhibitors," *Molecules 2021, Vol. 26, Page 5335*, vol. 26, no. 17, p. 5335, Sep. 2021, doi: 10.3390/MOLECULES26175335.

[26] S. B. Park *et al.*, "Anti-diabetic and anti-inflammatory effect of a novel selective 11β-HSD1 inhibitor in the diet-induced obese mice," *Eur J Pharmacol*, vol. 721, no. 1–3, pp. 70–79, Dec. 2013, doi: 10.1016/J.EJPHAR.2013.09.052.

[27] Z. Chuanxin *et al.*, "Progress in 11β-HSD1 inhibitors for the treatment of metabolic diseases: A comprehensive guide to their chemical structure

diversity in drug development," *Eur J Med Chem*, vol. 191, p. 112134, Apr. 2020, doi: 10.1016/J.EJMECH.2020.112134.

[28] D. F. Swaab, A. M. Bao, and P. J. Lucassen, "The stress system in the human brain in depression and neurodegeneration," *Ageing Res Rev*, vol. 4, no. 2, pp. 141–194, May 2005, doi: 10.1016/J.ARR.2005.03.003.

[29] M. J. Meaney *et al.*, "Individual differences in hypothalamic-pituitary-adrenal activity in later life and hippocampal aging," *Exp Gerontol*, vol. 30, no. 3–4, pp. 229–251, May 1995, doi: 10.1016/0531-5565(94)00065-B.

[30] V. Rajan, C. R. W. Edwards, and J. R. Seckl, "11 beta-Hydroxysteroid dehydrogenase in cultured hippocampal cells reactivates inert 11-dehydrocorticosterone, potentiating neurotoxicity," *Journal of Neuroscience*, vol. 16, no. 1, pp. 65–70, Jan. 1996, doi: 10.1523/JNEUROSCI.16-01-00065.1996.

[31] O. A. Ajilore and R. M. Sapolsky, "In vivo Characterization of 11β-Hydroxysteroid Dehydrogenase in Rat Hippocampus Using Glucocorticoid Neuroendangerment as an Endpoint," *Neuroendocrinology*, vol. 69, no. 2, pp. 138–144, Feb. 1999, doi: 10.1159/000054412.

[32] S. Gregory *et al.*, "11β-hydroxysteroid dehydrogenase type 1 inhibitor use in human disease-a systematic review and narrative synthesis," *Metabolism*, vol. 108, Jul. 2020, doi: 10.1016/J.METABOL.2020.154246.

[33] A. Anderson and B. R. Walker, "11β-HSD1 inhibitors for the treatment of type 2 diabetes and cardiovascular disease," *Drugs*, vol. 73, no. 13, pp. 1385–1393, Sep. 2013, doi: 10.1007/S40265-013-0112-5.

[34] J. S. Scott, F. W. Goldberg, and A. V. Turnbull, "Medicinal chemistry of inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)," *J Med Chem*, vol. 57, no. 11, pp. 4466–4486, Jun. 2014, doi: 10.1021/JM4014746.

[35] M. Cunliffe, "Who are the marine fungi?," *Environ Microbiol*, vol. 25, no. 1, pp. 131–134, Jan. 2023, doi: 10.1111/1462-2920.16240.

[36] A. S. Gladfelter, T. Y. James, and A. S. Amend, “Marine fungi,” *Current Biology*, vol. 29, no. 6, pp. R191–R195, Mar. 2019, doi: 10.1016/J.CUB.2019.02.009.

[37] I. Bhatnagar and S. K. Kim, “Immense Essence of Excellence: Marine Microbial Bioactive Compounds,” *Marine Drugs 2010, Vol. 8, Pages 2673-2701*, vol. 8, no. 10, pp. 2673–2701, Oct. 2010, doi: 10.3390/MD8102673.

[38] J. Silber, A. Kramer, A. Labes, and D. Tasdemir, “From Discovery to Production: Biotechnology of Marine Fungi for the Production of New Antibiotics,” *Mar Drugs*, vol. 14, no. 7, p. 137, Jul. 2016, doi: 10.3390/MD14070137.

[39] A. Pandey, “Pharmacological significance of marine microbial bioactive compounds,” *Environ Chem Lett*, vol. 17, no. 4, pp. 1741–1751, Dec. 2019, doi: 10.1007/S10311-019-00908-7/TABLES/3.

[40] L. Xu, W. Meng, C. Cao, J. Wang, W. Shan, and Q. Wang, “Antibacterial and Antifungal Compounds from Marine Fungi,” *Marine Drugs 2015, Vol. 13, Pages 3479-3513*, vol. 13, no. 6, pp. 3479–3513, Jun. 2015, doi: 10.3390/MD13063479.

[41] P. Bhadury, B. T. Mohammad, and P. C. Wright, “The current status of natural products from marine fungi and their potential as anti-infective agents,” *J Ind Microbiol Biotechnol*, vol. 33, no. 5, pp. 325–325, May 2006, doi: 10.1007/S10295-005-0070-3.

[42] M. Prata, C. De, and S. Esteves, “ANTICANCER ACTIVITY OF MARINE-DERIVED FUNGI EXTRACTS AND ISOLATED COMPOUNDS IN HUMAN CANCER CELL LINES”.

[43] D. Mitra *et al.*, “Evolution of bioinformatics and its impact on modern bio-science in the twenty-first century: Special attention to pharmacology, plant science and drug discovery,” *Computational Toxicology*, vol. 24, p. 100248, Nov. 2022, doi: 10.1016/J.COMTOX.2022.100248.

[44] C. G. Wermuth, B. Villoutreix, S. Grisoni, A. Olivier, and J. P. Rocher, “Strategies in the Search for New Lead Compounds or Original Working

Hypotheses," *The Practice of Medicinal Chemistry: Fourth Edition*, pp. 73–99, Aug. 2015, doi: 10.1016/B978-0-12-417205-0.00004-3.

[45] C. G. Wermuth, B. Villoutreix, S. Grisoni, A. Olivier, and J. P. Rocher, "Strategies in the Search for New Lead Compounds or Original Working Hypotheses," *The Practice of Medicinal Chemistry: Fourth Edition*, pp. 73–99, Jan. 2015, doi: 10.1016/B978-0-12-417205-0.00004-3.

[46] M. Nitulescu, T. Alves de Oliveira, M. Pires da Silva, E. Habib Bechelane Maia, A. Marques da Silva, and A. Gutterres Taranto, "Virtual Screening Algorithms in Drug Discovery: A Review Focused on Machine and Deep Learning Methods," *Drugs and Drug Candidates 2023, Vol. 2, Pages 311-334*, vol. 2, no. 2, pp. 311–334, May 2023, doi: 10.3390/DDC2020017.

[47] C. G. Wermuth, D. Aldous, P. Raboisson, and D. Rognan, "The Practice of Medicinal Chemistry: Fourth Edition," *The Practice of Medicinal Chemistry: Fourth Edition*, pp. 1–458, Aug. 2015, doi: 10.1016/C2012-0-03066-9.

[48] P. C. Agu *et al.*, "Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management," *Scientific Reports* |, vol. 13, p. 13398, 123AD, doi: 10.1038/s41598-023-40160-2.

[49] P. H. M. Torres, A. C. R. Sodero, P. Jofily, and F. P. Silva-Jr, "Key Topics in Molecular Docking for Drug Design," *Int J Mol Sci*, vol. 20, no. 18, Sep. 2019, doi: 10.3390/IJMS20184574.

[50] R. M. Abreu, H. J. Froufe, M. J. R. Queiroz, and I. C. Ferreira, "MOLA: a bootable, self-configuring system for virtual screening using AutoDock4/Vina on computer clusters," *J Cheminform*, vol. 2, no. 1, p. 10, Dec. 2010, doi: 10.1186/1758-2946-2-10.

[51] D. A. Gschwend, A. C. Good, and I. D. Kuntz, "Molecular Docking Towards Drug Discovery," *JOURNAL OF MOLECULAR RECOGNITION*, vol. 9, pp. 175–186, 1996, doi: 10.1002/(SICI)1099-1352(199603)9:2.

[52] A. Lauria, M. Ippolito, and A. M. Almerico, "Molecular docking approach on the Topoisomerase I inhibitors series included in the NCI anti-cancer

agents mechanism database," *J Mol Model*, vol. 13, no. 3, pp. 393–400, Mar. 2007, doi: 10.1007/S00894-006-0159-2/FIGURES/10.

[53] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: a review," *Biophys Rev*, vol. 9, no. 2, p. 91, Apr. 2017, doi: 10.1007/S12551-016-0247-1.

[54] G. M. Morris *et al.*, "Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function," *J Comput Chem*, vol. 19, no. 14, p. 16391662, 1639, doi: 10.1002/(SICI)1096-987X(19981115)19:14.

[55] "YASARA - Yet Another Scientific Artificial Reality Application." Accessed: Oct. 29, 2024. [Online]. Available: https://www.yasara.org/

[56] "Zentrum für Bioinformatik: Universität Hamburg - Proteins Plus Server." Accessed: Oct. 29, 2024. [Online]. Available: https://proteins.plus/

[57] S. K. Deshmukh, V. Prakash, and N. Ranjan, "Marine fungi: A source of potential anticancer compounds," *Front Microbiol*, vol. 8, no. JAN, p. 2536, Jan. 2018, doi: 10.3389/FMICB.2017.02536/FULL.

[58] R. C. Calhelha *et al.*, "New trends from fungi secondary metabolism in the pharmaceutical industry," *Natural Secondary Metabolites: From Nature, Through Science, to Industry*, pp. 823–850, Feb. 2023, doi: 10.1007/978-3-031-18587-8_26.

[59] S. K. Deshmukh, M. K. Gupta, V. Prakash, and M. Sudhakara Reddy, "Mangrove-Associated Fungi: A Novel Source of Potential Anticancer Compounds," *Journal of Fungi 2018, Vol. 4, Page 101*, vol. 4, no. 3, p. 101, Aug. 2018, doi: 10.3390/JOF4030101.

Annex I

Marine Fungi Library

Number	Smiles	Source	Reference
1	CC(C)C[C@@H](C(=O)NC1=CC=CC=C1C(=O)O)NC(=O)[C@@H](CC2=CC=CC=C2)N(C)C(=O)[C@H](C(C)C)N	*Simplicillium obclavatum* EIODSF 020e	[57]
2	CC[C@@H](C)[C@@H](C(=O)N(C)[C@@H](CC1=CC=C(C=C1)O)C(=O)N[C@@H](CC(C)C)C(=O)OC)NC(=O)C	*Simplicillium obclavatum* EIODSF 020e	[57]
3	CC[C@@H](C)[C@@H](C(=O)N(C)[C@@H](CC1=CC=C(C=C1)O)C(=O)O)NC(=O)C	*Simplicillium obclavatum*	[57]

		EIODSF 020e	
4	CC[C@@H](C)[C@@H](C(=O)N(C)[C@@H](CC1=CC=CC=C1)C(=O)O)NC(=O)C	*Simplicillium obclavatum* EIODSF 020e	[57]
5	C[C@@H]1[C@@H]([C@@H](C2=C(O1)C(=O)C3=C(C2=O)C=C(C=C3)O)O)O	*Acaromyces ingoldii* FS 121	[57]
6	C[C@@H]1[C@@H]([C@@H](C2=C(O1)C(=O)C3=C(C2=O)C=CC=C3O)O)O	*Acaromyces ingoldii* FS 121	[57]
7	C[C@]12C=C[C@H]([C@]3([C@H]1[C@@H](C=C4[C@@H]3CC(=O)OC4)OC2=O)C)O	*Aspergillus wentii* SD-310	[57]

8	C[C@]1(CCCC2=C3CC[C@]4([C@H](CO[C@H]4C3=C(C=C21)O)O)C)CO.C[C@]1(CCCC2=C3CC[C@]4([C@H](CO[C@H]4C3=C(C=C21)O)O)C)CO	*Aspergillus wentii* SD-310	[57]
9	C[C@H]1C2=NC3=C(C=CC=C3O)C(=O)N2C4=CC=CC=C4C(=O)N1	*Aspergillus westerdijkiae* SCSIO 05233	[57]
10	[C-]#[N+]/C(=C\C1=CC=C(C=C1)O)/C(=C/C2=CC=C(C=C2)O)/[N+]#[C-]	*Penicillium commune* SD-118	[57]
11	CC(C1=NC2=CC=CC=C2C(=O)N1)O	*Penicillium commune* SD-118	[57]
12	CC(C)(C=C)[C@@]12C=C(C(=O)N\3[C@]1(NC(=O)/C3=C\C4=CN=CN4)N(C5=CC=CC=C25)OC)O	*Penicillium commune* SD-118	[57]

13	C[C@@H]([C@H]1C=C(C(=O)O1)CC(=O)C)O	*Aspergillus* sp. 16-02-1	[57]
14	C[C@H]1C=C(C(=O)O1)CC[C@H](C)O	*Aspergillus* sp. 16-02-1	[57]
15	C[C@@H]([C@H]1C=C(C(=O)O1)[C@@H]([C@@H](C)O)O)O	from *Aspergillus* sp. 16-02-1	[57]
16	C[C@@H]([C@H]1C=C(C(=O)O1)[C@H]([C@H](C)O)O)O	from *Aspergillus* sp. 16-02-2	[57]
17	C[C@@H]([C@H]1C=C(C(=O)O1)[C@@H]([C@@H](C)O)OC)O	from *Aspergillus* sp. 16-02-3	[57]
18	C[C@@H]([C@H]1C=C(C(=O)O1)CC(C)O)O	from *Aspergillus* sp. 16-02-4	[57]

19	C[C@H](C=C(CO)C=CC(=O)C)O	from *Asper gillus* sp. 16-02-5	[57]
20	C[C@@H]1[C@H](C=C(C(=O)O1)[C@@H]([C@@H](C)O)OC)O	from *Asper gillus* sp. 16-02-6	[57]
21	C[C@@H]1[C@H](C=C(C(=O)O1)C[C@H](C)O)O	from *Asper gillus* sp. 16-02-7	[57]
22	C[C@H](/C=C/C(=C/[C@H](C)O)/CO)O	from *Asper gillus* sp. 16-02-8	[57]
23	C[C@@H](/C=C/C(=C/[C@H](C)O)/CO)O	from *Asper gillus* sp. 16-02-9	[57]
24	CC1=C(C=C(OC1=O)C[C@H](C)O)O	from *Asper gillus* sp. 16-02-10	[57]

25	C[C@@]12[C@@H]3[C@@H](C=C4COC(=O)C=C4[C@]3([C@H]5[C@@H]([C@@H]1O)O5)C)OC2=O	*Aspergillus dimorphicus* SD317	[57]
26	C[C@]12C[C@H](C[C@]3([C@@H]1[C@@H](C=C4C2=CC(=O)OC4)OC3=O)C)O	*Aspergillus dimorphicus* SD318	[57]
27	C[C@H](/C=C/[C@H](C)C(C)C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@]24C=C[C@@]5([C@@]3(CC[C@@H](C5)O)C)OO4)C	*Paecilomyces lilacinus* ZBY-1.	[57]
28	CCCCCCCCCCCC/C=C/[C@H](C(=O)N[C@@H](CO[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)[C@@H](/C=C/CC/C=C(\C)/CCCCCCCCC)O)O	*Paecilomyces lilacinus* ZBY-1.	[57]
29	CCCCCCCCCCCCCC[C@H](C(=O)N[C@@H](CO[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)[C@@H](/C=C/CC/C=C(\C)/CCCCCCCCC)O)O	*Paecilomyces lilacinus* ZBY-1.	[57]
30	CCCCCCCCCCCCCC/C=C/[C@H](C(=O)N[C@@H](CO[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)[C@@H](/C=C/CC/C=C(\C)/CCCCCCCCC)O)O	*Paecilomyces*	[57]

		lilacinus Z BY-1.	
31	CCCCCCCCCCCCCCCC[C@H](C(=O)N[C@@H](CO[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)[C@@H](/C=C/CC/C=C(\C)/CCCCCCCCC)O)O)O	*Paecilomyces lilacinus* Z BY-1.	[57]
32	CC1=C[C@@H]([C@H]2[C@]([C@]13CC4=C(O3)C(=C(OC4=O)C)C)(CC[C@@H]5[C@@]2(C=CC(=O)C5(C)C)C)C)O	*Penicillium* sp.	[57]
33	CC1=CC[C@H]2[C@]([C@]13CC4=C(O3)C(=C(OC4=O)C)C)(CC[C@@H]5[C@@]2(C=CC(=O)C5(C)C)C)C	*Penicillium* sp.	[57]
34	COC1=C(C(=C(C2=C1O[C@H]3[C@@]2(C4=CC=CC=C4N3)OC)OC)OC)C5=CNC6=CC=CC=C65	*Paecilomyces variotii* EN -291	[57]
35	CC(C)[C@@]1(C2=NC3=C(C=CC=CO3)C(=O)N2[C@H](C(=O)N1)CC4=CC=CC=C4)OC	*Paecilomyces variotii* EN -292	[57]
36	C[C@@]1(CCC[C@]2([C@H]1[C@@H](C=C3[C@@]2(COC3=O)O)OC(=O)C4=CC=C(C=C4)[N+](=O)[O-])C)CO	*Aspergillus*	[57]

		ochraceus Jcma1F17	
37	CC1=CC2=C(C(=C1)O)C(=O)C3=C(C2=O)C=C(C=C3O)OC	*Microsporum* sp	[57]
38	C[C@H]1C(=O)N/C(=C\C2=C(NC3=CC=CC=C32)C(C)(C)C=C)/C(=O)N1	*Microsporum* sp.	[57]
39	C[C@]12C=C[C@H]([C@]3([C@H]1[C@@H](C=C4C3=CC(=O)OC4)OC2=O)C)O	*Aspergillus wentii* EN-48	[57]
40	C[C@]12C[C@H]3[C@H](O3)[C@]4([C@@H]1[C@@H](C=C5C2=CC(=O)OC5)OC4=O)C	*Aspergillus wentii* EN-48	[57]
41	C1=C[C@@H]([C@]2([C@H]3[C@]1(C1=CC(=O)OCC1=C[C@H]3OC2=O)C)C)O	*Aspergillus wentii* EN-48	[57]
42	C1C2=CC=CC=C2O[C@@]13C(=O)N/C(=C\C4=CC=CC=C4O)/C(=O)N3	*Penicillium brocae* MA-231	[57]

43	C1[C@@H]2[C@H](C=C[C@@H]([C@H]2N3[C@]14C(=O)N5[C@@H]6[C@H](C=CC=C6C[C@]5(C3=O)SS4)O)O)O	*Penicillium brocae* MA-231	[57]
44	CCCCCCCCCC1=C(C(=C(C(=C1Br)O)Br)O)C(=O)OCC	*Lasiodiplodia* sp. 318	[57]
45	CO[C@@H]1CC(=O)C2=C3[C@@]14OC5=C6C(=C(C=C5)O)C(=O)[C@H]([C@H]([C@]6(O4)OC3=CC=C2)O)Cl	*A. Ilicifolius*	[57]
46	CO[C@@H]1CC(=O)C2=C3[C@@]14OC5=C6C(=C(C=C5)O)C(=O)[C@H]([C@H]([C@]6(O4)OC3=CC=C2)OC)Cl	*A. Ilicifolius*	[57]
47	C1[C@H]([C@]23C4=C(C1=O)C=CC=C4O[C@@]5(O2)[C@H]6[C@H](O6)C(=O)C7=C(C=CC(=C57)O3)O)O	*A. Ilicifolius*	[57]
48	C1C[C@]23C4=C(C1=O)C=CC=C4O[C@@]5(O2)[C@H]6[C@H](O6)C(=O)C7=C(C=CC(=C57)O3)O	*A. Ilicifolius*	[57]
49	C1=CC2=C3C(=C1)O[C@@]45[C@H]6[C@H](O6)C(=O)C7=C(C=CC(=C74)O[C@@]3(O5)C=CC2=O)O	*A. Ilicifolius*	[57]
50	C1C[C@]23C4=C([C@H]1O)C=CC=C4O[C@@]5(O2)[C@H]6[C@H](O6)C(=O)C7=C(C=CC(=C57)O3)O	*A. Ilicifolius*	[57]

51	C[C@H]1CCC/C=C\[C@@H](C=C[C@@H](/C=C/C(=O)O1)O)OC	*Pestalotiopsis microspore*	[57]
52	C[C@H]1CCC/C=C\[C@@H](/C=C\C(=O)CCC(=O)O1)OC	*Pestalotiopsis microspore*	[57]
53	C[C@H]1CCC[C@H](/C=C/C=C\[C@@H](/C=C/C(=O)O1)O)O	*Pestalotiopsis microspore*	[57]
54	C[C@H]1CCC[C@H](/C=C\C=C\[C@H](/C=C/C(=O)O1)O)O	*Pestalotiopsis microspore*	[57]
55	CC=C[C@@H]1[C@H]2C[C@H](CC[C@@H]2[C@]([C@H]3[C@H]1C(=O)C4=C(O3)C(=CNC4=O)C5=CC=C(C=C5)O)(C)O)C	*Campylocarpon sp.* HDN13-307	[57]
56	C/C=C/[C@@H]1[C@H]2C[C@H](CC[C@@H]2C(=C[C@H]1C(=O)C3=C(C(=CNC3=O)C4=CC=C(C=C4)O)O)C)C	*Campylocarpon sp.* HDN13-308	[57]

57	C[C@]1([C@@H](C[C@H]2[C@H]([C@H]1O)C(=O)C3=C(C2=O)C(=CC(=C3)OC)O)O)O	*Stemphylium globuliferum*	[57]
58	C[C@]1([C@@H]([C@H](C2=C([C@H]1O)C(=O)C3=C(C2=O)C(=CC(=C3)OC)O)O)O)O	*Stemphylium globuliferum*	[57]
59	C[C@@]1(CC2=C(C[C@H]1O)C(=O)C3=C(C2=O)C=C(C=C3O)OC)O	*Stemphylium globuliferum*	[57]
60	C[C@]1([C@@H]([C@H](C2=C([C@H]1O)C(=O)C3=C(C2=O)C(=CC(=C3C4=C(C=C(C5=C4C(=O)C6=C(C5=O)[C@@H]([C@H]([C@@]([C@@H]6O)(C)O)O)O)O)OC)OC)O)O)O)O	*Stemphylium globuliferum*	[57]
61	CC(=C)[C@@H]1[C@@H]([C@@H]2[C@]3(O2)C4CC[C@]5([C@@]([C@]4(CCC3O1)C)(C6=C7[C@H](O5)OC([C@H]8C[C@H]9[C@@]8(C1=C7C(=CC(=C1CC9=C)Cl)N6)O)(C)C)C)O)O	*Mucor irregularis* QEN-189	[57]

62	CC(=C)[C@@H]1[C@@H]([C@@H]2[C@]3(O2)C4CC[C@]5([C@@]([C@]4(CCC3O1)C)(C6=C7[C@H](O5)OC([C@H]8C[C@H]9[C@@]8(C1=C7C(=CC(=C1CC9=C)Cl)N6)O)(C)C)C)OC)O	*Mucor irregularis* QEN-189	[57]
63	CC(=C)[C@@H]1[C@@H]([C@@H]2[C@@]3(O2)[C@@H](O1)CC[C@]4([C@]3(CC[C@@H]5[C@@]4(C6=C7[C@H]5OC([C@H]8C[C@H]9[C@@]8(C1=C7C(=CC(=C1CC9=C)Cl)N6)O)(C)C)C)O)C)O	*Mucor irregularis* QEN-189	[57]
64	CC(=C)[C@@H]1[C@@H]([C@@H]2[C@@]3(O2)[C@@H](O1)CC[C@]4([C@]3(CC[C@@H]5[C@@]4(C6=C7[C@H]5OC([C@H]8C[C@H]9[C@@H]8C1=C(CC9=C)C=CC(=C17)N6)(C)C)C)O)C)O	*Mucor irregularis* QEN-189	[57]
65	CC(=C)[C@@H]1[C@@H]([C@@H]2[C@@]3(O2)[C@@H](O1)CC[C@]4([C@]3(CC[C@@H]5[C@@]4(C6=C7[C@H]5OC([C@H]8C[C@H]9[C@@H]8C1=C7C(=CC(=C1CC9=C)Cl)N6)(C)C)C)O)C)O	*Mucor irregularis* QEN-189	[57]
66	c1cccc2c1c1c([nH]2)[C@]2([C@H](C1)CC[C@@H]1[C@@]2(CC[C@H]2C1=C[C@H]([C@H](C2)C(C)(C)O)O)C)C	*Mucor irregularis* QEN-189	[57]
67	C(O)[C@H](C[C@@H]([C@H](CC[C@@H]([C@@H]([C@H](C[C@@H]([C@H]([C@@H](C[C@@H](C[C@@H](CC)C)CO)C)O)C)C)O)C)O)C)C	*Pestalotiopsis clavispora*	[57]
68	CC1=CC(=O)C2=C(O1)C(=C(C=C2O)OC)[C@@H]3C[C@H](C(=O)O3)OC	*Rhytidhysteron rufulum*	[57]

69	CC1=CC(=O)C2=C(O1)C(=C(C=C2O)OC)[C@@H](C[C@H](C(=O)OC)OC)OC	*Rhytidhysteron rufulum*	[57]
70	CC1=CC(=O)C2=C(O1)C(=C(C=C2O)OC)[C@H](C[C@H](C(=O)OC)OC)OC	*Rhytidhysteron rufulum*	[57]
71	CC1=CC(=O)C2=C(O1)C(=C(C=C2O)OC)[C@H](C[C@H](C(=O)O)OC)OC	*Rhytidhysteron rufulum*	[57]
72	CC(C)[C@@H]1C(=O)N([C@H](C(=O)O[C@@H](C(=O)N([C@H](C(=O)O[C@@H](C(=O)N([C@H](C(=O)O1)CC2=CC=CC=C2)C)C(C)C)CC3=CC=CC=C3)C)C(C)C)CC4=CC=CC=C4)C	*Fusarium* sp.	[57]
73	CC1=C(C=C2C(=C1C=C)CC[C@@H]([C@@]2(C)CCC(=O)OC)C(=C)C)O	*Ceriops tagal*	[57]
74	CC=C[C@@H]1[C@@H](C=C2[C@@H](O1)[C@@H](OC2=O)C=CC)O	*Acremonium strictum*	[57]
75	C1C[C@@H](C([C@@]23[C@]1([C@H]([C@@](C[C@@H]2O3)(OCc1c(c2c(c(c1O)C=O)[C@H](OC2=O)OC)OC)C)C(=O)O)C)(C)C)Cl	*Acremonium strictum*	[57]
76	CCCCCCCC1C2=C(C(=C(C=C2C(=O)O1)O)O)O	*Acremonium strictum*	[57]
77	CCCCCCCC(C1=C(C=C(C=C1O)O)CC(=O)OC)OC	*Dothiorella* sp.	[57]
78	CCCCCCCC(=O)C1=C(C=C(C=C1O)O)CC(=O)OCC	*Dothiorella* sp.	[57]

79	C[C@H](/C=C/C(C)C(C)C)[C@H]1C[C@@H](C2=C3C=C[C@@]45C[C@H](CC[C@@]4([C@]3(CC[C@]12C)OO5)C)O)O	*Aspergillus niger* MA-132	[57]
80	C[C@H](/C=C/C(C)C(C)C)[C@H]1C[C@H](C2=C3C=C[C@@]45C[C@H](CC[C@@]4([C@]3(CC[C@]12C)OO5)C)O)O	*Aspergillus niger* MA-132	[57]
81	C[C@]12CCC[C@]3([C@@H]1[C@@H]([C@H]([C@H]4C2=CC(=O)OC4)O)OC3=O)C	*Aspergillus terreus* (No. GX7-3B)	[57]
82	CC[C@H](C)[C@H]1C(=O)N[C@H](C(=O)N([C@H](C(=O)O[C@@H](C(=O)N2CCC[C@H]2C(=O)N[C@H](C(=O)N1C)CO)CC3=CC=CC=C3)C)C)CC4=CC=C(C=C4)OCC=C(C)C	*Bionectria ochroleuca*	[57]
83	C[C@H]1C(=O)O[C@@H](C(=O)N2CCC[C@H]2C(=O)N[C@H](C(=O)N([C@H](C(=O)N[C@H](C(=O)N1C)CC3=CC=C(C=C3)OCC=C(C)C)C(C)C)C)CO)CC4=CC=CC=C4	*Bionectria ochroleuca*	[57]
84	CC([C@]12C(=O)N3[C@@H]4[C@]([C@@H]([C@@]3(C(=O)N1C)SS2)O)(C5=CC=CC=C5N4)[C@]67[C@@H]([C@]89C(=O)N([C@](C(=O)N8[C@H]6NC1=CC=CC=C71)(SS9)C(C)O)C)O)O	*Bionectria ochroleuca*	[57]
85	CC[C@H](C)[C@H]1C(=O)N[C@H](C(=O)N([C@H](C(=O)OC(C(=O)N2CCC[C@H]2C(=O)N[C@H](C(=O)N1C)C)CC3=CC=CC=C3)C)C)CC4=CC=C(C=C4)OCCC(=C)C	*Bionectria ochroleuca*	[57]
86	CC[C@H](C)[C@@H](C(=O)N[C@@H](CC1=CC=C(C=C1)OCC=C(C)C)C(=O)O)N(C)C(=O)[C@H](CO)NC(=O)[C@@H]2CCCN2C(=O)C(CC3=CC=CC=C3)O	*Bionectria ochroleuca*	[57]

87	CN1[C@H](C(=O)N2[C@H](C1=O)C[C@@]3([C@H]2NC4=CC=CC=C43)[C@@]56C[C@H]7C(=O)N([C@H](C(=O)N7[C@@H]5NC8=CC=CC=C68)CC9=CC=CC=C9)C)CC1=CC=CC=C1	No·Gx-3a	[57]
88	CC(C)C(=O)OC[C@@]1([C@@H](CC2C[C@@]23C1[C@H](C(=O)C4=C3CC[C@]([C@@H]4O)(C)C=C)O)OC(=O)C)C	*Eutypella* sp. FS46	[57]
89	CC/C=C\[C@@H]([C@@H](C1=C(C(=O)[C@@]2(O1)[C@H]([C@@](NC2=O)(C(=O)C3=CC=CC=C3)OC)O)C)O)O	*Aspergillus* sp. (BRF 030)	[57]
90	CCC(/C=C/[C@@H](C1=C(C(=O)[C@@]2(O1)C([C@@](NC2=O)(C(=O)C3=CC=CC=C3)OC)O)C)O)O	*Aspergillus* sp. (BRF 030)	[57]
91	CC(=C[C@H]1C2=C(C[C@@H]3N1C(=O)[C@@H]4CCCN4C3=O)C5=C(N2)C=C(C=C5)OC)C	*Aspergillus* sp. (BRF 030)	[57]
92	CC(=C[C@H]1c2c([C@@H]([C@]3(N1C(=O)[C@@H]1CCCN1C3=O)O)O)c1c([nH]2)cc(cc1)OC)C	Aspergillus sp. (BRF 030)	[57]
93	CC1(C(=O)N2[C@@H](N1C=O)[C@]3(C[C@H](C(=O)O3)N4C=NC5=CC=CC=C5C4=O)C6=CC=CC=C62)C	*Neosartorya fischeri*	[57]
94	CC1(C(=O)N2[C@H](N1)[C@]3(C[C@H](C(=O)O3)N4C=NC5=CC=CC=C5C4=O)C6=CC=CC=C62)C	*Neosartorya fischeri*	[57]

95	CC(C)[C@H]1C2=NC3=CC=CC=C3C(=O)N2[C@@H](C(=O)N1)CC4=CNC5=CC=CC=C54	*Neosartorya fischeri*	[57]
96	CC(=NCC(C)(C)O)NC1=CC=CC=C1C(=O)O	*Penicillium paneum* SD-44	[57]
97	C1=CC=C(C(=C1)C(=O)O)NC=NC2=CC=CO2	*Penicillium paneum* SD-44	[57]
98	CC1=CC2=C(C(=C1)O)OC3=C(C(=C(C=C3)CC=C(C)C)OC)C(=O)OC2	*Penicillium* sp. ZLN29	[57]
99	CC1=CC2=C(C(=C1)O)OC3=C(C(=C(C=C3)[C@H](CC(C)C)O)OC)C(=O)OC2	*Penicillium* sp. ZLN29	[57]
100	C[C@H]1C[C@@H]([C@@H]2[C@@]([C@](C=C[C@]2(C1)O)(C)O)(C)C(=O)CCO)O	*Aspergillus sulphureus* KMM 4640	[57]
101	c1(c(c(cc2c1C(=O)c1c(C2=O)cc(c(c1O)[C@H](CCCCC)O)O)OC)C)O	*Aspergillus* sp. SCSIO F063	[57]

102	CC1(C(CCC2C1=C(C(=O)C3=CC(CC[C@]23O)C=C)O)O)C	*Eutypella scoparia* F S26	[57]
103	C[C@@]1(CC[C@]2(C(=C1)C(=O)C(=C3[C@@]2([C@@H](CCC3(C)C)O)C)O)O)C=C	*Eutypella scoparia* F S26	[57]
104	C[C@@]1(C[C@H]([C@]2(C(=C1)C(=O)[C@]3([C@@]4([C@@]2(CCCC4(C)C)CO3)O)O)O)O)C=C	*Eutypella scoparia* F S26	[57]
105	C[C@H]/1C/C=C/[C@H]2[C@H]3[C@](O3)([C@H]([C@@H]4[C@@]2(C(=O)/C=C/C(=O)[C@@H](/C(=C1)/C)OC(=O)C)C(=O)N[C@H]4CC5=CC=CC=C5)C)C	*Arthrinium arundinis* Z SDS1-F3	[57]
106	CC1=CC(=C(C(=O)O1)C[C@@H]2C(=C)CC[C@H]3[C@]2(CC[C@@H]4[C@@]3(CC[C@@H](C4(C)C)OC(=O)C)C)C)O	*Neosartorya laciniosa* (KUFC 7896)	[57]
107	CC(=CCN1C2=C(C=CC(=C2)OC)C3=C1[C@@H](N4C(=O)[C@@H]5CCCN5C(=O)[C@@]4(C3=O)O)C=C(C)C)C	*Neosartorya laciniosa* (	[57]

		KUFC 7896)	
108	CC(C)CCC[C@@](C)(C1=C(C=C(C=C1)COC2=C(C=CC(=C2)CO)[C@](C)(CCCC(C)C)O)O)O	*Aspergillus* sp	[57]
109	CC(C)CCC[C@@](C)(C1=C(C=C(C=C1)CC2=CC(=C(C=C2CO)O)[C@](C)(CCCC(C)C)O)O)O	*Aspergillus* sp	[57]
110	C(#C/C=C/B)C#CC#C/C=C/C#CCC#CC#CC(=O)NC(=N)CC	*Xestospongia* sp.	[57]
111	C[C@@H]1C2=CC(=C(C(=C2C(=O)N1C3=C(C=C(C=C3)OC)OCC=C(C)C)OC)C)OC/C=C(\C)/CCC=C(C)C	*Stachylidium*	[57]
112	C[C@H]1C2=CC(=C(C(=C2C(=O)N1C3=C(C=C(C=C3)OC)OCC=C(C)C)OC)C)OC/C=C(\C)/CCC=C(C)C	*Stachylidium*	[57]
113	CC[C@H](C)C(C(C)(/C=C/C1=CC2=CC(=O)[C@]([C@@H]([C@H]2CO1)O)(C)O)O)O	*Penicillium sclerotiorum* M-22	[57]
114	CCC(C)C(C(C)(C(/C=C/C1=CC2=C(C(=O)[C@]([C@@H](C2CO1)O)(C)O)Cl)O)O)O	*Penicillium sclerotiorum* M-22	[57]

115	C1=C(OC(=C1)C=O)CO	*Penicillium chrysogenum* HGQ6	[57]
116	CCOC(=O)C1([C@H]2CC3=C([C@@H]4[C@H]2[C@](O1)(C5=C4OC6=CC(=CC(=C6C5=O)O)C)C(=O)OC)C(=O)C7=C(C=C(C=C7O3)C)O)O	*Penicillium purpurogenum*	[57]
117	CC1=C(C=C(C=C1O)O)[C@@H](C)C(=O)C	*Penicillium purpurogenum* G59	[57]
118	CCCCCCC(C)C(=O)C1=C([C@@]2([C@@H]([C@@H]3C4=C(O2)C=C(C(=C4[C@@H]([C@H](O3)C)C)C)O)C)N(C1=O)C)O	*Penicillium citrinum*	[57]
119	C=C(C)C[C@@H]1NC(=O)[C@]23[C@H]1[C@@H]1[C@@](C[C@@H]2CC(=C)C#CCC(=C=C(C3=O)S#C)O)(C)C1	*Aspergillus* sp.	[57]
120	CCC(C)[C@@H]1C(=O)N([C@@H](C(=O)NC2=CC=CC=C2C(=O)N[C@@H](C(=O)N1)C)[C@H](C3=CC=CC=C3)O)C	*Aspergillus terreus* SCSGAF0162	[57]
121	CC1=CC(=C(C(=C1)O[C@@H]2C(=O)C(=C(C(=O)[C@@]2(C)OC)O)C)C)O	*Aspergillus aculeatus*	[57]
122	CC1=CC(=C(C2=C1C(=O)[C@@]3(O2)C(=O)C(=C(C(=O)[C@@]3(C)OC)O)C)C)O	*Aspergillus aculeatus*	[57]

123	CC1=CC(=CC(=C1)OC2=C(C(=CC(=C2)O)C)CC=C(C)C)O	*Aspergillus versicolor*	[57]
124	CC1=CC(=CC(=C1)OC2=C(C(=CC(=C2)O)C)CC(=O)C(C)C)O	*Aspergillus versicolor*	[57]
125	C[C@H]1C[C@H]2[C@@H](OC3=C([C@@H]2[C@H]([C@@H]1O)C)C(=O)NC=C3C4=CC=C(C=C4)O)C	*Chaunopycnis* sp. (CMB-MF028)	[57]
126	CC1=CC(=C2C3=C1C(=CC(=C3[C@H](OC2=O)C(=O)OC)O)O)O	*Penicillium purpurogenum* G59	[57]
127	C12C3C(CC(C1C(c(c2C(=O)OC3)O)C)O)O	*Penicillium purpurogenum* G59	[57]
128	CC(=C)C[C@H]1CN2CCC[C@H]2C(=O)N1	*Penicillium purpurogenum* G59	[57]

129	CC1=CC(=C2C(=C1)OC3=C(C2=O)C[C@H](S3)[C@](C[C@H](C(=O)OC)O)(C(=O)OC)O)O	*Penicillium oxalicum* SCSGAF 0023	[57]
130	COC(=O)[C@H]1[C@@H](C=CC2=C1C(=O)C3=C(C=C(C=C3O2)CO)O)O	*Neosartorya fischeri* strain 1008F1	[57]
131	C[C@]12CCC(=C1[C@@H]3CC(C[C@@H]3[C@@H]2O)(C)C)C(=O)O	*Chondrostereum* sp.	[57]
132	C/C/1=C\[C@H]([C@@H]([C@@H]2[C@H]3CC([C@@]3([C@H](C1)OC(=O)C)O[C@H]2O)(C)C)O)O	*Ascotricha* sp. ZJ-M-5	[57]
133	C/C/1=C/[C@H]([C@@H]([C@@H]2[C@H]3CC([C@@]3([C@H](C1)OC(=O)C)O[C@H]2O)(C)C)OC)O	*Ascotricha* sp. ZJ-M-5	[57]
134	C1CCC([C@]2([C@@]31[C@@]1(C(=C[C@](C[C@H]1O)(C=C)C)C(=O)[C@@]2(O)OCC3)O)O)(C)C	Fungal strain HS-1	[57]
135	COC1=C(C2=C(C=C1)C=C(C(=O)O2)NC(=O)C3=NO[C@H]4[C@@H](C=C[C@H]([C@]4(C3)O)Cl)O)OC	*Trichoderma virens*	[57]

136	CC1=CC(=C2C(=C1)OC3=C(C2=O)C[C@H]([C@]3(C(=O)OC)O)SC[C@@H](C(=O)OC)O)O	*Penicillium purpurogenum* G59	[57]
137	CC(C)CC1=NC=C(N(C1=O)O)C(C(C)C)O	(strain CF07002) of the genus *Aspergillus*	[57]
138	CC(C)CC1=CN=C(C(=O)N1O)CC(C)C	(strain CF07002) of the genus *Aspergillus*	[57]
139	CC1=CC(=CC(=C1C(=O)C2=C3C4=C(C(=CC(=C4)O)O)C(=O)C5=C(C=C(C(=C35)OC2=O)C)O)O)O	*Aspergillus glaucus*	[57]
140	C1CC(=O)C2=C([C@H]1O)C=CC=C2O	*Aspergillus fumigatus*	[57]
141	CC1=CC(=C2C(=C1)OC3=C(C2=O)C[C@H](S3)C(CC(=O)OC)(C(=O)OC)O)O	*Penicillium oxalicum* S	[57]

		CSGAF 0023	
142	C[C@@]1(CC2=C(C[C@H]1O)C(=C3C(=O)C=C(C(=O)C3=C2O)OC)O)O	*Nigrospora* sp. No. 1403	[57]
143	OC(=O)c1ccccc1n1c(nc2ccccc2c1=O)[C@@H]1CC[C@H](N1)N	*Penicillium aurantiogriseum*	[57]
144	COC(=O)C1=CC=CC=C1N2C(=NC3=CC=CC=C3C2=O)[C@@H]4CCC(=N4)N	*Penicillium aurantiogriseum*	[57]
145	C1=CC=C2C(=C1)C(=O)N3C4=CC=CC=C4C(=O)N[C@H](C3=N2)CCC(=O)N	*Penicillium aurantiogriseum*	[57]
146	CC(C)C(C)C=CC(C)C1CCC2C1(CCC3C2=CC=C4C3(CCC(C4)O)C)C	*Pestalotiopsis* spp.	[57]
147	CC[C@H](CC[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CCC4[C@@]3(CCC(=O)C4)C)C)C(C)C	*Pestalotiopsis* spp.	[59]
148	CC[C@H](CC[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CCC4=CC(=O)CC[C@]34C)C)C(C)C	*Pestalotiopsis* spp.	[59]

149	C/C=C/[C@@H]1C=C[C@@H]2C[C@@H](CC[C@H]2[C@]1(C)/C(=C\3/C(=O)[C@@H](N(C3=O)C)CO)/O)C	*Fusarium heterosporum* and a *Phoma* sp.	[59]
150	C/C=C\[C@@H]([C@@H](C1=C(C(=O)[C@@]2(O1)[C@H]([C@@](NC2=O)(C(=O)C3=CC=CC=C3)OC)O)C)O)O	*Aspergillus sydowii*	[58]
151	C[C@@H]1[C@H]2C=CC=C[C@@H]([C@@H]1[C@H]3[C@H](C=CC(=O)O3)OC(=O)C)O2	*Diaporthe* sp.	[58]
152	COC1=CC(=CC2=C1C(=O)C3=C(C2=O)C=C(C=C3O)CO)O	*E. amstelodami*	[58]
153	CCCCCCC1=C(C=C(C(=C1C=O)O)CC=C(C)C)O	*Eurotium* sp. SF-5989	[58]
154	CCCCC(C)/C=C(\C)/C(=O)O[C@@H]1CC[C@@H]([C@@]2(C1=CC(=O)[C@@H](C2)C(=C)C=O)C)C(=O)O	*Xylaria* sp.	[58]
155	CC(C)([C@H](C(=O)O)N)S	*Penicillium commune*	[58]
156	CC(=O)O[C@@H]1[C@H](C2=C([C@H]([C@]1(C)O)O)C(=O)C3=C(C2=O)C(=CC(=C3)OC)O)O	*Alternaria, Ampelomyces,*	[58]

		Stemphylium botryosum	
157	C[C@H]1C/C=C\[C@H]2[C@@H](C(=C)[C@H]([C@@H]3[C@@]2(C(=O)N[C@H]3CC4=CC=CC=C4)OC(=O)C/C=C(\C1=O)/C)C)O	*Arthrinium arundinis* ZSDS1-F3	[58]