INVESTIGATION *IN VITRO* AND *IN SILICO* EFFECTS OF SOME METAL COMPLEXES ON ESSENTIAL DIGESTIVE ENZYMES AND CHOLINESTERASES

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Master Thesis
FOOD TOXICOLOGY

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April 2024

I certify that in my opinion the thesis submitted by Raneem Mamoun ALI NOUR titled "INVESTIGATION *IN VITRO* AND *IN SILICO* EFFECTS OF SOME METAL COMPLEXES ON ESSENTIAL DIGESTIVE ENZYMES AND CHOLINESTERASES" is fully adequate in scope and in quality as a thesis for the degree of Master of Science.

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Raneem Mamoun ALI NOUR

ABSTRACT

Master Thesis

INVESTIGATION *IN VITRO* AND *IN SILICO* EFFECTS OF SOME METAL COMPLEXES ON ESSENTIAL DIGESTIVE ENZYMES AND CHOLINESTERASES

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Metal complexes are an important coordination system. They are chemical substances that carry a central atom, usually a metal ion, attached to a group of molecules or ions known as ligands or complexing agents. In the human body, some metals, including nickel, copper, cobalt, iron, and zinc, play a vital role. These metals might be present in small quantities within biological systems. The bipyridine ligand is often utilized metal-binding agent because of the high redox robustness and ease of modification. As uncharged ligand, bipyridine produces ionic compounds with metal cations is employed in the design and construction of metal bipyridine complexes. Bipyridine are heterocyclic compounds that consist of two pyridine rings; there are six types of bipyridines with different positions of the linkage, and more studied were the group of 2, 2'-bipyridines, which are fixed by the bond between the carbon atoms neighbouring the nitrogen atom of each pyridine. Within this study we discover the effect

synthesized metal complex on α-glucosidase, lipase pancreatic, acetylcholinesterase, and butyrylcholinesterase. A metal complex with ligands was synthesized, we used these synthesized substances. The effects of these metal complexes with known molecular weights on enzyme activities were determined and IC_{50} values were calculated for those showing inhibition effects.

Keyword: α-glucosidase, Lipase, Acetylcholinesterase, Butyrylcholinesterase, Inhibition, Docking.

Science Code : 701.3.019

ÖZET

Yüksek Lisans Tezi

BAZI METAL KOMPLEKSLERİN TEMEL SİNDİRİM ENZİMLERİ VE KOLİNESTERAZLAR ÜZERİNE *İN VİTRO* VE *İN SİLİKO* ETKİLERİNİN İNCELENMESİ

Raneem Mamoun ALI NOUR

Karabük Üniversitesi
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Metal kompleksleri önemli bir koordinasyon sistemidir. Bunlar, ligandlar veya kompleks oluşturucu maddeler olarak bilinen bir grup molekül veya iyona bağlı, genellikle bir metal iyonu olan merkezi bir atom taşıyan kimyasal maddelerdir. İnsan vücudunda nikel, bakır, kobalt, demir ve çinko gibi bazı metaller hayati bir rol oynar. Bu metaller biyolojik sistemlerde küçük miktarlarda mevcut olabilir. Bipiridin ligandı, yüksek redoks sağlamlığı ve modifikasyon kolaylığı nedeniyle sıklıkla metal bağlama maddesi olarak kullanılır. Yüksüz ligand olarak bipiridin, metal katyonlarla iyonik bileşikler üretir ve metal bipiridin komplekslerinin tasarımında ve yapımında kullanılır. Bipiridin, iki piridin halkasından oluşan heterosiklik bileşiklerdir. Bununla birlikte bağlantı pozisyonlarına sahip altı tip bipiridin vardır ve her piridin azot atomuna komşu karbon atomları arasındaki bağla sabitlenen 2, 2'-bipiridin grubu üzerinde daha çok çalışma yapılmıştır. Bu çalışma kapsamında sentezlenen metal

kompleksinin α-glukozidaz, pankreatik lipaz, asetilkolinesteraz ve butirilkolinesteraz üzerindeki etkileri incelendi. Ligand ile metal kompleksleri sentezlendi ve sentezlenen bu maddeler çalışmada kullanıldı. Molekül ağırlıkları bilinen bu metal komplekslerinin enzim aktiviteleri üzerindeki etkileri belirlendi ve inhibisyon etkisi gösterenler için IC_{50} değerleri hesaplandı.

Anahtar Kelimeler: α-glukozidaz, Lipaz, Asetilkolinesteraz, Bütirilkolinesteraz, İnhibisyon, Doking.

Bilim Kodu **:** 701.3.019

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOLS

μg	: Microgram
g	: Gram
L	: Litre
mL	: Millilitre
mg	: Milligram
M	: Molar
mM	: Millimolar
nm	: Nanometre
μL	: Microliter
μM	: Micromolar
μg	: Microgram

ABBREVIATIONS

WHO : World Health Organization
IC_{50} : Half-maximal inhibitory concentration
pH : potential of hydrogen
AChE : Acetylcholinesterase
BChE : Butyrylcholinesterase
PL : Pancreatic lipase
DNA : Deoxyribonucleic acid
ROS : Reactive oxygen species
Cu : Copper
Co : Cobalt
Ni : Nickel
Bpy : 2,2′-Bipyridine
AD : Alzheimer's disease
DM : Diabetes Milltuos
ChAT : Choline acetyltransferase
ChE-Is : Cholinesterase inhibitors
CHT1 : choline transporter
CNS : Central nervous system
APP : Amyloid precursor protein
VAChT : Vesicular acetylcholine transporter

SECTION 1

INTRODUCTION

Coordination metal complexes play a crucial role in biological systems due to their covalent bonds, which regulate between the ligands and the central atom and identifying their availability of coordination sites based on oxidation state. Complexes of metals are chemical substances composed of central atoms or ions that are associated to a group of molecules or ions, knowns as ligands or complexing agents. These complexes, including iron, cobalt, nickel, copper, and zinc, have been found effective in the human system over thousands of years. They can adhere to the sulfur, nitrogen, and selenium atoms of the cysteine, histidine, and seleno cysteine residues in proteins inhibit enzymes, and provide therapeutic benefits [1]. Metal compounds are widely used in physical and chemical science due to their electronic and stereochemical properties, and have shown promise in treating diseases like diabetes, ulcers, rheumatoid arthritis, and cardiovascular conditions, many important metal ions in our meals are essential in variable amounts, actually their clinical significance is still not widely recognized, which might be related to our greater awareness of persons and family health [2]. In some processes, metal ions interact with ligands as oxidizing and reducing agents in biological systems. Iron is the most important metal in biological systems. It is involved in oxygen transport in the blood and tissues, respiration processes, and influences various processes related to the metabolism of proteins, lipids, carbohydrates, and nucleic acids. [3].

An adult requires 250 ml of pure oxygen per minute during rest, which is transported by the metal complex system known as heme. This system allows oxygen to leave the blood as it reaches the tissues. A heme group is a metal complex with iron as the central metal atom, which binds to and releases molecular oxygen. [3].

Cobalt is crucial for the synthesized form of vitamin B12 (cobalamin, Figure 1.1.). It has an indirect effect on DNA synthesis and the generation of myelin, an insulation covering found surround neurons and used in the production of red blood cells, its deficiency can result in pernicious anaemia and peripheral nervous system disorders [4,5]. It is used as catalysts in reactions. Cobalt complexes have attracted the scientific community's interest due to their therapeutic efficiency as tumour imaging agents, anticancer, antimycobacterial, antiviral, antiparasitic, and antithrombotic capabilities [5].

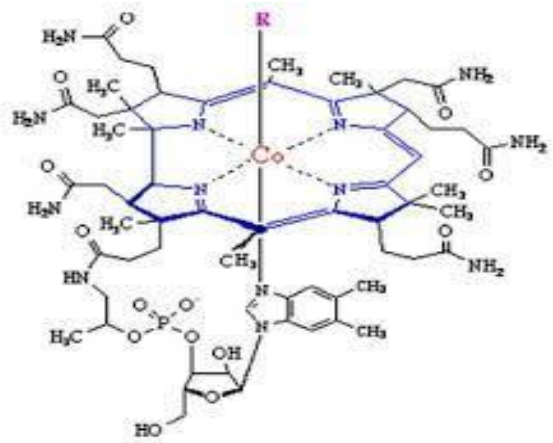


Figure 1.1. Chemical composition of vitamin B12 [4].

Approximately a third of all known proteins with a metal cofactors including iron, zinc, and copper all play important roles in biological processes for dioxgen, protein structure stabilization, enzymes catalysis, and critical reactions [6, 7].

The coordination field between copper ion and ligands may lead in complexes of various stereochemistry and geometry. These complexes may be found in a variety of forms, the most frequent of which are mononuclear, binuclear, and multinuclear [6, 7, 8].

Copper (Cu) is a crucial trace element necessary for life. It ranks as the third most abundant transition metal in biological systems, after iron and zinc. As a d9 ion, copper is present in a wide array of chemical compounds with diverse structures, including mononuclear, binuclear, and polynuclear species. These compounds exhibit unique geometries, magnetic properties, and spectroscopic features [8]. Copper serves as a cofactor in over 30 enzymes crucial for maintaining cellular activities in humans and animals. These include cytochrome c oxidase, dopamine ß hydroxylase, tyrosinase,

lysyl oxidase, and zinc-copper dependent superoxide dismutase. Moreover, copper significantly impacts electron transfer during cellular respiration, energy production, iron oxidation, pigment and connective tissue formation, as well as neurotransmitter and antioxidant synthesis, owing to the roles these enzymes play in the human system. It is used biologically in a variety of roles, including detoxification of reactive oxygen species (ROS), mitochondrial respiration, and connective tissue development. Copper is distinct as it can be oxidized to form Cu(I), Cu(II), Cu(III), and Cu(IV), especially in gas phase oxidation (Figure 1.2.)[8]. These oxidation states have been demonstrated to manifest diverse pharmacological effects, such as antidiabetic, anticancer, antitumor, anti-inflammatory, antiulcer, and antimicrobial activity [6-8]. Sources of copper encompass vegetables, cereals, meat, fish, poultry, and legumes. The typical daily intake of copper from the average human diet supplies around 1,400 µg/day for men and 1,100 µg/day for women, primarily absorbed in the upper small intestine. Copper is distributed in the body in quantities ranging from 50 mg to 120 mg. This micronutrient is predominantly present in the liver, brain, and bones, with lesser amounts found in the heart, pancreas, and kidneys.

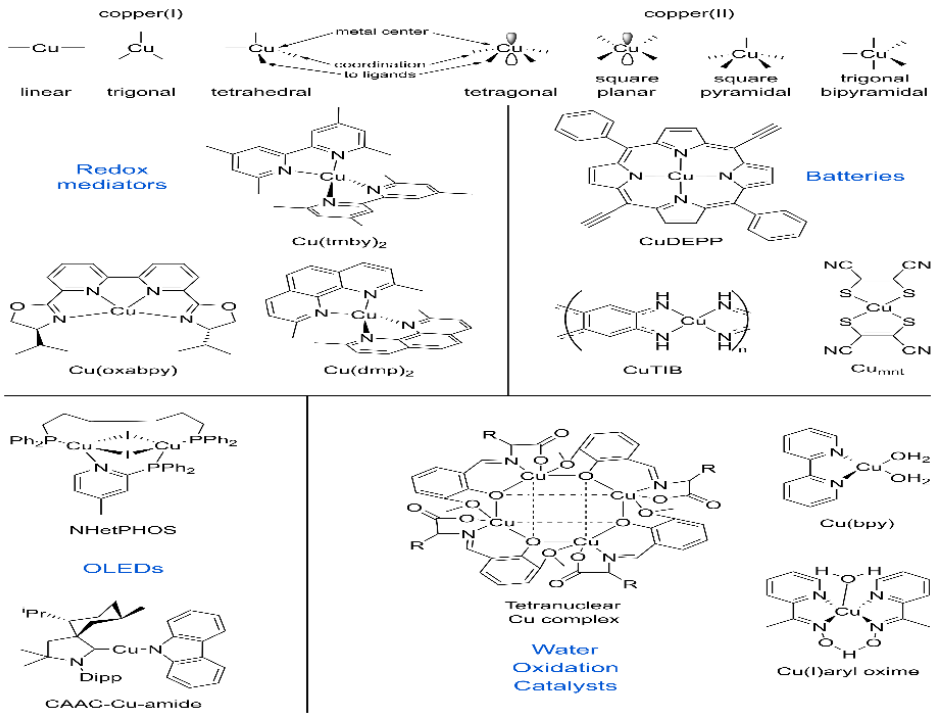


Figure 1.2. Classification of copper [8].

Nickel, is a stable transition metal, is utilized in catalysis to create C-C bonds via cross and symmetric interactions with ligands with different chemical and physical characteristics. It impacts metabolic processes, suppressing enzymatic activity, photoelectron transport, and chlorophyll production, and is required for growth and development in microorganism, primitive, eukaryotes, and plants [9]. Recent studies have focused on several nickel-containing enzymes, such as urease, methylcoenzyme M reductase, CO dehydrogenase, Ni-superoxide dismutase, glyoxalase, acireductone dioxygenase, lactate racemase, prolyl cis-trans isomerase, and [NiFe] hydrogenase. [10]. Metal complexes bind with ligands in a process categorized as either anions or neutral molecules that act as electron pair donors (Lewis bases). These ligands can be connected in two manners: either as a single donor atom to the metal, referred to as monodentate, or with two or more heteroatoms, termed bidentate or polydentate. [11].

According to the site of action, metals provide opportunities for use as therapeutic agents. Schiff bases are a category of ligands in coordination chemistry derived from pyridoxal and amino acids. Reactions catalysed by ligand metal have been one of the main research topics in the field of coordination chemistry [11]. The creation of mixed ligand complexes has resulted in their utilization as active catalysts in industrially significant reactions, such as hydrogenation, hydroformylation, oxidative hydrolysis of olefins, and methanol carboxylation [12]. Bipyridines have reshaped our comprehension of the thermodynamics and kinetics associated with metal ions. Renowned for their exceptional coordination properties and stability, bipyridines serve as both heterocyclic neutral ligands and bidentate ligands. The development of novel and precise synthetic techniques for these isomers has sparked increased interest across various domains, including catalysis, photochemistry, electrochemistry, and supramolecular chemistry [12]. The most common coordination formula for 2,2’-

bipyridine is that of a chelating bidentate ligand, wherein nitrogen atoms bind to the same metal centre [M(bpy)3]n+ (Figure 1.3.) [13].

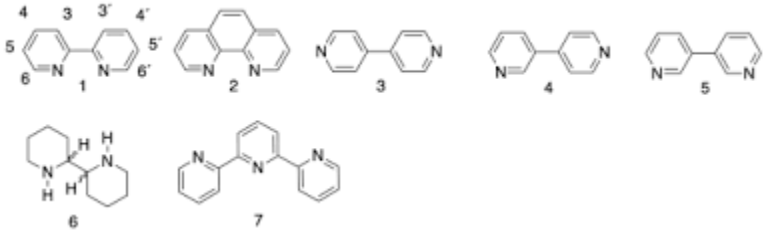


Figure 1.3. System for assigning numbers to 2,2'-bipyridine (bpy) and homologous N-heterocyclic ligands [13].

Subsequently, enzymes play a vital role in all kind of metabolic pathways reaction involve in body growth, blood clotting, healing process, food digestion, the mechanism of reproduction, the DNA replication process, the transcription process, the synthesis of protein from amino acids, and process of signal transduction. It is linked with many diseases. Enzyme action is to bind with substrate and form enzyme-substrate complexes, which are then converted to final product (Figure 1.4.) [3].

The enzymes are a biological catalyst essential for the occurring of biological reactions, classified as Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, and Lygases, and for greater efficiency require different conditions than chemical catalysts for triggering, which show a metal target based on drug design. The enzymes have high specificity and catalyse reactions faster than chemical catalysts. If a molecule accelerates the activity of the enzyme, it is called an enzyme activator, and if the molecule decreases the activity of the enzyme, it is called an enzyme inhibitor. However enzymatic catalysis is remarkable in that it permits high-quality goods to be

produced at a lower cost while reducing environmental hazards with the addition of a significant advantage to humans in numerous types of activity [14].

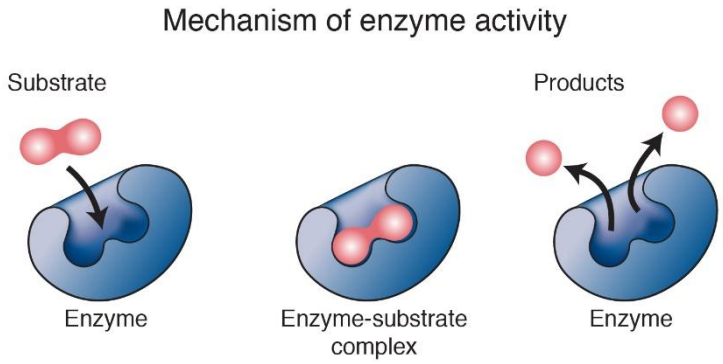


Figure 1.4. Enzymatic catalysis mechanism [3].

Enzyme inhibitors are molecules, either simple or complex, that can be inorganic or organic in nature. They function by reducing or halting the activity of enzymes, which in turn affects the rate of enzymatic reactions. There are several types of enzyme inhibitors are reversible, irreversible and allosteric (Figure 1.5.) [14].

They have been used in many fields of medicinal chemistry, pharmacology, biochemistry, and biotechnology enzyme inhibitors are a strong research sector and whose main goal is design, discovery and improvement of enzyme inhibitors. There are number of cases disease that treated by enzyme inhibitors [14].

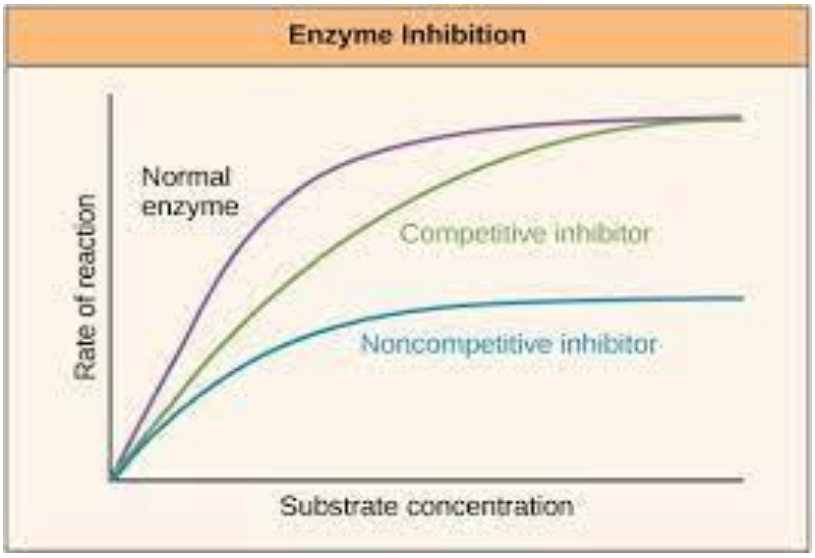


Figure 1.5. Enzyme inhibition process [14].

The human digestive system performs a vital role in the digestion and absorption of food. During the digestion process, the structure of the food is broken down through complicated interaction of chemical and mechanical processes. There is a digestive juices contain enzymes promote the breakdown of proteins, carbohydrates and fats. Therefore, this research intends to investigate the efficiency of various crucial enzymes on the therapeutic human body.

Diabetes Mellitus disease: Diabetes, a metabolic disorder marked by elevated blood sugar levels (hyperglycemia), is becoming more widespread globally and is projected to become the seventh leading cause of death by 2030, as per the World Health Organization (WHO). [15]. The onset of diabetes occurs when the pancreas fails to produce enough insulin or when the body is unable to effectively use the insulin it produces. This disorder is classified into four types based on the underlying pathogenic mechanisms that lead to hyperglycaemia: Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus, and Non-classical causes of DM [16]. Individuals with diabetes may experience various complications such as atherosclerosis, cardiac dysfunction, retinopathy, and neuropathy [16,17]. Nutrients and functional foods have been shown to improve dyslipidaemia and reduce the risk of cardiovascular disease [17]. Treatment of diabetes involves the synthesis of certain metals interacting with specific ligands to produce metal-ligand complexes, which play a significant role in metallotherapy [17].

α-amylase enzyme: In 1894, the enzyme alpha amylase was identified from a fungus source and has since been utilized to treat digestive problems [18, 19]. A group of industrial enzymes makes up about 30% of the world enzyme production. Despite the fact that they may be obtained from a variety of sources, including microorganisms (Bacillus subtilis and Bacillus mesentericus) [18, 19], microbial enzymes, animal and plant, most amylase are metalloenzyme that are require calcium ions for their action. The primary enzyme involved in starch digestion is pancreatic alpha-amylase, which catalyzes the hydrolysis of α-1,4 glycosidic linkages present in starch, amylopectin, amylose, glycogen, and numerous maltodextrins [15,18,19,20]. Another essential enzyme is alpha-glucosidase, also known as maltase, which facilitates the final stage

of carbohydrate digestion, particularly starch, by breaking down 1,4-α bonds to produce glucose [18,19,20,21].

In individuals with normal glucose metabolism, large starch molecules cannot pass through the blood-brain barrier directly as glucose. Instead, α-amylase breaks down these starch molecules into smaller sugar fragments, enabling their passage through the blood-brain barrier [18, 19, 20, 21]. Excessive conversion of starch to sugars can lead to elevated blood glucose levels. In response, insulin prompts cells to utilize excess sugar and store it as glycogen for energy [15]. Elevated blood glucose levels may result from heightened amylase enzyme activity coupled with insulin deficiency or resistance. Several studies are investigating the inhibition of amylase enzyme activity to reduce hyperglycaemia [18, 19]. However, severe inhibition of pancreatic alpha-amylase may result in abnormal bacterial fermentation of undigested carbohydrates in the colon, leading to stomach cramps and gas [15].

Some anti-diabetic drugs function by inhibiting carbohydrate digestion and absorption. These include:

- Acarbose (BAY g 5421), a microbial-derived inhibitor and the first commercially available alpha-glucosidase inhibitor for diabetic treatment, which suppresses alpha-amylase, sucrase, and maltase activity.
- Miglitol, derived from 1-deoxynojirimycin, has a significant inhibitory effect on sucrose, glucoamylase, and isomaltase activities.
- Voglibose, a bacterial-derived inhibitor that inhibits the actions of α-glucosidase, sucrase, isomaltase, and maltase.

Alzheimer's disease (AD): AD is a progressive neurological disease that causes cognitive impairment over time. One of the earliest indications of Alzheimer's disease is short-term memory loss, it is known as dementia, was first described more than 100 years ago. Nowadays, over 24 million people worldwide suffer from dementia, and the global incidence of dementia is estimated to double every 20 years, reaching 42 million people in 2020 and 81 million people in 2040 [22].

Patients progressively deteriorate and experience additional symptoms, such as chronic memory impairment, disorientation, language and circadian rhythm

disruptions, emotional instability, loss of motor abilities, and eventually, death. The characteristics of Alzheimer's disease are associated with the accumulation of neurofibrillary tangles and amyloid plaques [22].

Histopathological studies of Alzheimer's disease in both humans and animal models have identified amyloid plaques and neurofibrillary tangles in multiple brain regions, notably the basal forebrain, frontal lobe, hippocampus, and cerebral cortex. Amyloid plaques consist of β-amyloid peptides (Aβ), which are formed from the enzymatic cleavage of amyloid precursor protein (APP). Interestingly, the amount of cortical plaques does not directly correlate with cognitive decline in Alzheimer's disease, suggesting that additional factors contribute to disease progression. Tau, a protein associated with microtubules, becomes hyperphosphorylated and forms oligomers, leading to the development of neurofibrillary tangles. Cholinergic neurons are extensively distributed throughout the central nervous system (CNS) [22]. Cholinergic neurons are predominantly located in the spinal cord, hindbrain, medial habenula, mesopontine region, basal forebrain, striatum, olfactory tubercle, and the islands of Calleja complex. These neurons innervate nearly all areas of the brain. [22].

Acetylcholine esterase (AChE) enzyme: Published data indicate that acetylcholine (ACh) plays a crucial role in brain function. However, maintaining the balance among various neurotransmitter systems, including acetylcholine (ACh), norepinephrine, dopamine, γ-aminobutyric acid (GABA), serotonin, and glutamate, is essential for proper brain function. [23].

Acetylcholine (ACh) is synthesized from choline and acetyl-coenzyme A (acetyl-CoA) in the cytosol of cholinergic presynaptic neurons through the action of the enzyme choline acetyltransferase (ChAT). After its production, ACh is transported into synaptic vesicles by the vesicular acetylcholine transporter (VAChT) [22,23].

When a presynaptic neuron depolarizes, it triggers the exocytosis of ACh from synaptic vesicles into the synaptic cleft. In the synaptic cleft, ACh can bind to either nicotinic or muscarinic receptors, leading to a stimulatory or inhibitory response. The enzyme acetylcholinesterase (AChE) rapidly breaks down ACh in the synaptic cleft into acetate and choline. The released choline is then taken back up into the presynaptic

cholinergic neuron through the high-affinity choline transporter (CHT1, Figure 1.6) [22].

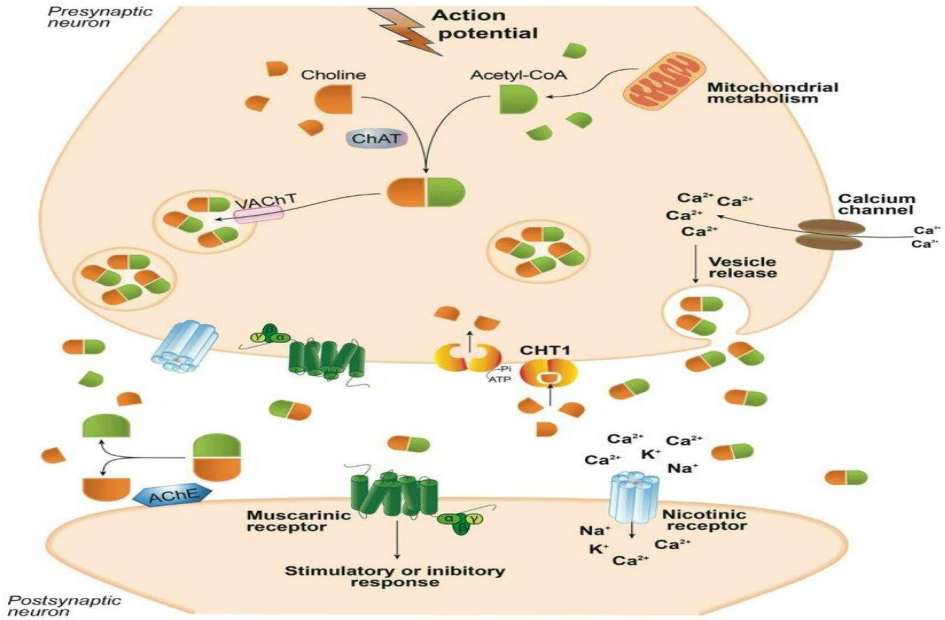


Figure 1.6. Figure illustrating acetylcholine neurotransmission in biology [22].

The hypothesis suggests that the acetylcholine biosynthetic enzyme the choline acetyltransferase (ChAT) is related in Alzheimer's disease brain regions, leading to the use of cholinesterase enzyme inhibition in Alzheimer's patients [23].

The mechanism of work for the cholinesterases, AChE and butyrylcholinesterase (BChE), hydrolytically breakdown ACh in the brain, inhibitors (ChE-Is) reduce neurotransmitter breakdown by boosting brain ACh levels and thereby improving inadequate brain cholinergic neurotransmission. ChE-Is were the first medications approved in the US and Europ of the specific indication for symptomatic treatment of AD [23].

Obesity: Obesity has been a significant health risk for many years, posing more than just a cosmetic issue. It disrupts normal physiological metabolism, leading to various physical, psychological, and social challenges. Obesity is a major risk factor for numerous diseases, including heart disease, hypertension, hyperlipidemia, diabetes,

and cancer. Individuals with obesity are at a higher relative risk for type 2 diabetes, gallbladder disease, and metabolic syndrome compared to those with normal weight. The condition typically arises from excessive fat consumption, physical inactivity, and unhealthy lifestyle choices. Preventing and treating obesity is crucial to reducing the morbidity and mortality associated with chronic metabolic diseases. Excess fat is stored in the liver and white adipose tissue, and its accumulation can cause non-alcoholic fatty liver disease and hypertrophy of white adipose tissue. The human body cannot directly utilize dietary fats, which must first be hydrolyzed for absorption. [24].

Obesity treatment typically involves a combination of short-term pharmacological interventions and surgical procedures, such as liposuction, alongside long-term strategies like calorie restriction and regular exercise. However, liposuction can result in uneven fat distribution and poses certain risks without significantly affecting subcutaneous fat. Therefore, current weight reduction approaches emphasize the use of appropriate short-term medications in conjunction with sustained dietary and exercise regimens.

Within the digestive system, lipases play a crucial role in fat digestion, including tongue lipase, gastric lipase, and pancreatic lipase. Gastric lipase is particularly important, as it regulates the secretion of pancreatic lipase and assists in the breakdown of fats during digestion. [24].

Pancreatic lipase: The most crucial enzyme for fatty acid absorption in the gut is pancreatic lipase (PL). This primary lipase, produced by the pancreas, hydrolyzes dietary lipids in the digestive tract, converting triacylglycerols in ingested oils into monoglycerides and free fatty acids. These monoglycerides and free fatty acids are then transported to and absorbed by enterocytes, which line the intestines. Once fat-containing food is digested by enzymes, the triglycerides are broken down by lipase into monoglycerides, glyceryl esters, and free fatty acids, with a higher yield of 1,2-glycolide and fatty acids.

Although lingual lipase contributes minimally to fat degradation, it can hydrolyze 50% to 70% of fat intake in newborns and young children. Following initial digestion, fats

are further broken down into free fatty acids and monoacylglycerol in the gastrointestinal tract and small intestine by gastric lipase (responsible for 10% to 30% of fat decomposition) and pancreatic lipase (responsible for 50% to 70% of fat decomposition). These processes eventually lead to the formation of cholesterol and lipoproteins in the body (Figure 1.7) [25].

Lipase inhibitors work by altering the conformation of gastric and pancreatic lipases, reducing their catalytic activity. By interacting with the active sites of these lipases in the stomach and small intestine, these inhibitors decrease the breakdown and absorption of dietary fats, such as triglycerides, thereby limiting fat accumulation and aiding in obesity management. After their action, lipase inhibitors are typically excreted along with the lipase they inhibit, which minimizes their long-term impact on the body.

Current weight loss medications fall into two main categories: peripheral lipase inhibitors and central appetite suppressants. Peripheral lipase inhibitors, such as orlistat, reduce fat absorption in the intestine. Central appetite suppressants, like fenfluramine and sibutramine, act on the central nervous system to decrease appetite. However, clinical studies have shown that these medications can cause adverse reactions, including headaches, dizziness, dry mouth, constipation, and insomnia. More concerning are the potential mental and cardiovascular side effects, which can significantly restrict their therapeutic use and even lead to the withdrawal of some medications. Given the incomplete safety profile of central appetite suppressants, there is a pressing need to develop new weight loss drugs with fewer side effects [24, 25].

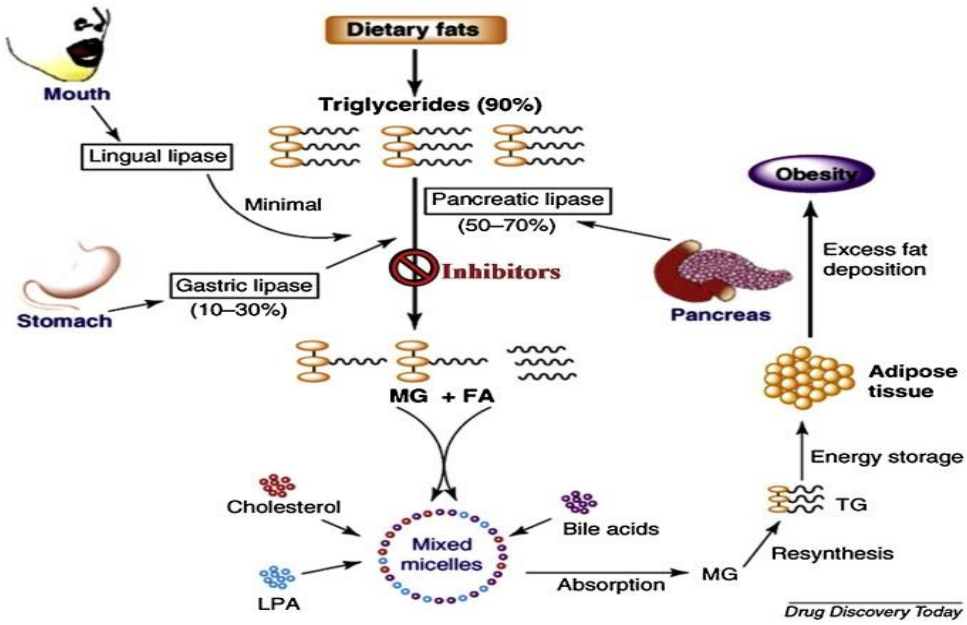


Figure 1.7 Biological pathways for lipid metabolism in humans [24].

SECTION 2

PURPOSE AND IMPORTANCE OF THE STUDY

2.1. BIPIRIDINE LIGANTS AND COORDINATION COMPOUNDS

Metallotherapy is an emerging therapeutic approach used to treat various diseases, including cancer, diabetes, rheumatoid arthritis, inflammatory conditions, and cardiovascular diseases. Metals make up about 0.03% of human body weight and exhibit diverse properties depending on their oxidation state, the quantity and type of coordinated ligands, and the coordination geometry of their complexes. Ligands primarily govern the reactivity of metals, but they also play crucial roles in mediating interactions with biological targets such as DNA, enzymes, and protein receptors. Transition metals, including chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), and copper (Cu), are particularly significant in biological systems. These metals are integral to various enzymes, influencing enzyme-catalyzed reactions by modulating electron flow within substrates or enzymes. Without the appropriate metal ion, certain metalloenzyme-catalyzed biological reactions would proceed very slowly. Metals assist in binding and orienting substrates relative to functional groups in the enzyme's active site, thereby facilitating these reactions. [28]. In 1888 was the first discovered of 2,2′-bipyridin until to the outbreak of the second global conflict in 1939. Coordination chemistry and analytical applications are explained and contextualized in the perspective of the more advanced characterisation methods that were accessible to chemists during this time period. Many of the "simple" 2,2′-bipyridine compounds reported in the early literature were later proven to have more complicated structures. Fritz Blau in 1888 was used copper (II) pyridine-2-carboxylate that lead loss of gases involving hydrogen cyanide and production of a distillate including pyridine and a new base with melting point 70 ^{o}C (Figure 2.1) [11].

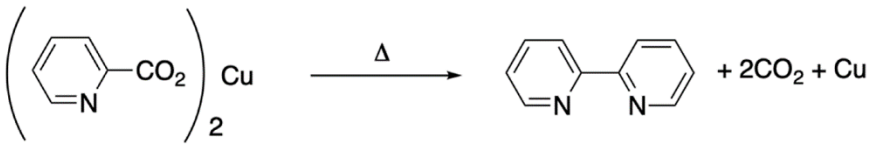


Figure 2.1. Fritz Blau initially synthesized 2, 2'-bipyridine through the dry distillation of copper (II) pyridine-2-carboxylate.

2.1.1. Ligand History

Schiff bases derived from salicylaldehydes are well-known for their polydentate ligand properties. Complexes of transition metals with nitrogen and oxygen donors are significant in agricultural, medicinal, and industrial chemistry. Schiff base complexes with the general formula [ML2]·2H2O have been synthesized, where M2+ represents Mn, Fe, Co, Ni, or Cu, and L is salicylidene p-aminoacetophenone. To understand the coordination of the Schiff base through nitrogen and oxygen, various techniques were employed, including elemental analysis, magnetic susceptibility measurements, molar conductivity, FTIR, and UV-Visible spectroscopy. The data suggest that the bonding of the metal ions (M (II)) to the ligand (L) results in the formation of an octahedral structure (Figure 2.2) [29, 30].

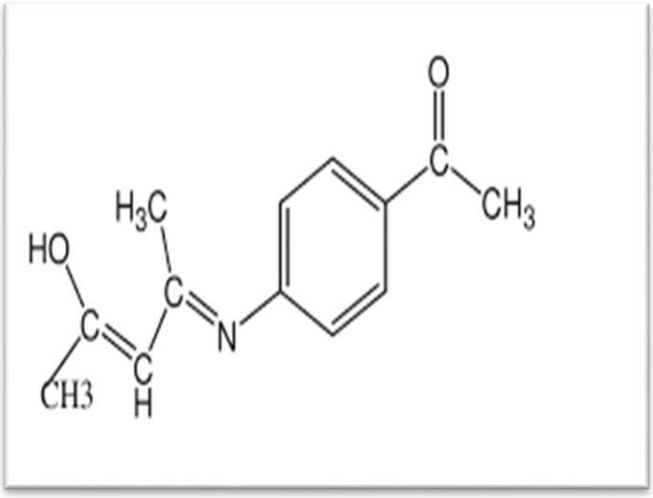


Figure 2.2. Structure of the ligand molecule [29].

2.1.2. Fact of Metal Complex with Enzyme

In some metal complexes, the metal center does not directly coordinate with the enzyme. Instead, the ligands serve as the physiologically active components responsible for protein binding or enzyme inhibition. The metal can undergo redox reactions that enhance these effects. In certain cases, the ligands act to shield a "naked" metal ion, which typically interacts with many ligands in a non-selective manner and is usually not bioactive. However, the metal is crucial for stabilizing or protecting the reactive center. Conversely, there are complexes where both the metal and the ligands are physiologically active, jointly contributing to the biological activity of the complex. In these complexes, the metal and ligands work synergistically to produce the desired therapeutic effects. [32, 33].

2.1.2.1. Metal and Enzyme Active Site

Jaouen successfully incorporated redox-active ferrocenyl groups into well-established pharmacological structures. By substituting a phenyl ring in hydroxytamoxifen (the active metabolite of tamoxifen, a potent drug used to treat hormone-dependent breast cancer), he developed a new class of drugs called hydroxyferrocifens. These ferrocifens show potential for treating tumors that lack estrogen receptors (Figure 2.3). Additionally, the inclusion of the metal fragment not only expands the therapeutic range but also tackles challenges related to acquired drug resistance. [31].

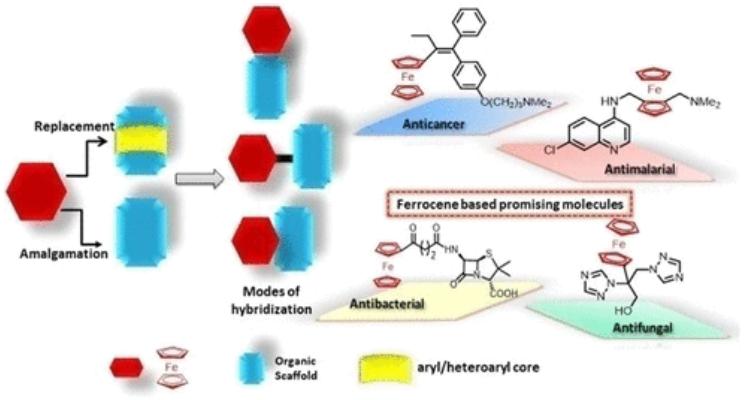


Figure 2.3. Mechanism of ferrocene [31].

A method was created for occupying 3D-space in enzyme active sites by employing metal pieces. They employed an inert metal as a framework to orient physiologically active ligands, resulting in metal complexes that function similarly to organic molecules. When compared to 2D organic structures, this enhanced coordination number and isomers allowed for improved inhibitor selectivity. However, pioneer compounds mimic staurosporine, a potent ATP-competitive protein kinase inhibitor, with an inert metal center. These metal-based mimics can undergo significant structural changes, altering auxiliary ligands surrounding the metal. The first complexes inhibited Pim-1, 43, but following structural changes, nanomolar and picomolar inhibitory characteristics were found. However, minor changes resulted in large variances in selectivity for distinct kinase family members. Slight structural changes in members of the kinase family can result in large alterations in selectivity, particularly with organic compounds. This method has been used to various metal centers such as platinum, osmium, iridium, and rhodium. Co-crystal structures of the enzyme's active site with staurosporine analogues have also been found. The link between ligand and active site, like ATP and staurosporine binding, persists independent of the kinase. The metal ion works as a glue, allowing for greater variety in the synthesis process and the identification of highly active enzyme inhibitors (Figure 2.4.) [32].

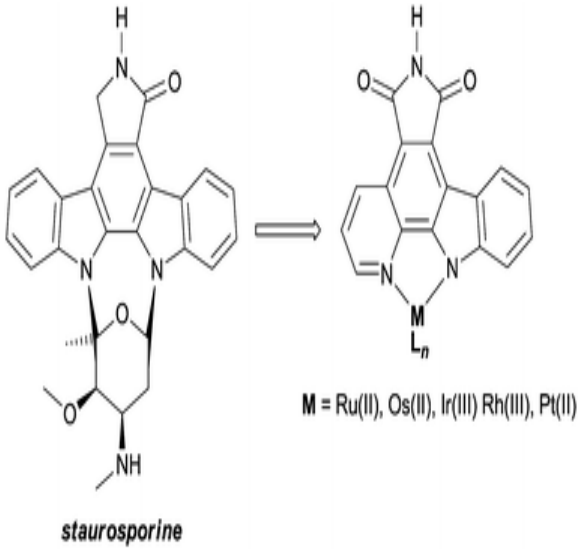
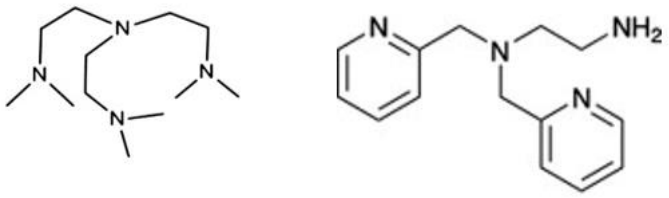


Figure 2.4. Taurosporine and metal-based analogs as protein kinase blockers [32].

Metal complexes can inhibit enzymes through various mechanisms. Traditional metal-based drugs often target DNA and bind with multiple proteins simultaneously. However, recent studies suggest that well-designed metal complexes can achieve higher selectivity than organic inhibitors and may serve as more effective inhibitors through direct coordination. These complexes hold significant promise as tools in chemical biology. Given the growing reliance on targeting specific enzymes and pathways in developing treatments for diseases like cancer, metal complexes with selective enzyme inhibition capabilities offer vast potential in medicinal chemistry. Future research might focus on non-toxic metals that are more biocompatible and possess enhanced pharmacological properties, especially when used as scaffolds. This direction could lead to the development of safer and more effective therapeutic agents for a wide range of disorders. [32].

2.1.2.2. The Consequences of Metal Complexes Formed With Ligands

The well-known tripodal tetradentate 4N ligands tris [2-(dimethylamino) ethyl] amine (Me6TREN, L1) and (2-aminoethyl) bis (2-pyridylmethyl) amine (uns-penp, L2) were synthesized and characterized by Tordin and colleagues as new catalysts for the homogeneous partial oxidation of alkanes (Figure 2.5). The only isolated L2 complex, the nickel derivative [Ni(L2)(CH3COO)][PF6] (complex 3), demonstrated limited stability under substrate oxidation conditions due to the fragility of ligand L2. Consequently, catalytic activity measurements focused on L1 metal complexes. The general formula for all L1 derivatives is [M(L1)(CH3COO)][PF6], where M can be cobalt(II), nickel(II), or copper(II). These complexes are pentacoordinate in the solid state, with only one acetate oxygen atom coordinated to the metal center. L1 cobalt and nickel complexes have been shown to catalyze the hydroxylation of cyclohexane and adamantane in the presence of m-CPBA as an oxidant, achieving good selectivity and high turnover numbers (TONs). While the cobalt derivative (complex 1) exhibits the highest TONs, the nickel complex (complex 2) is the most selective catalyst for partial alkane oxidation in homogeneous solution at room temperature. However, under identical reaction conditions, the corresponding copper L1 derivative (complex 4) shows no catalytic activity for alkane oxidation. [34].



L1 L2

Figure 2.5. The molecular structures of the ligands were employed [34]

In medicinal chemistry, inorganic chemistry can be categorized into two main areas. The first involves the use of ligands, either alone or in a protein-bound form, as therapeutic agents. The second focuses on metal-based drugs and imaging agents, where the central metal ion plays a crucial role in the mechanism of action. Transition metal complexes, characterized by their variable oxidation states due to an incomplete d-subshell, can interact with a wide range of negatively charged ions. These interactions make transition metal complexes particularly valuable for various medicinal and pharmacological applications [35].

Ajaz and co-workers synthesized a novel Schiff base derived from amantadine and investigated its transition metal complexes as potential inhibitors of alkaline phosphatase (ALP), α-amylase, and α-glucosidase. The Schiff base ligand HL, specifically (E)-2-((adamantan-1-ylimino) methyl)-6-allylphenol, was synthesized by condensing amantadine with 3-allyl-2-hydroxybenzaldehyde. The resulting ligand was then used to create its Zn (II), Co (II), Cr (III), and VO (IV) complexes under reflux conditions. A variety of spectroscopic and analytical techniques were employed to thoroughly characterize the synthesized compounds. These techniques included UV-Vis spectroscopy, 1H and 13C NMR, FT-IR, ESI-MS, thermal analysis, and single-crystal XRD. Additionally, molar conductance and elemental analysis confirmed the chemical nature of the synthesized substances. Based on the data, the researchers proposed octahedral geometry for the Cr (III) and Co(II) complexes, tetrahedral geometry for the Zn(II) complex, and square pyramidal geometry for the VO(IV) complex. In vitro inhibition tests for α-amylase and α-glucosidase were conducted to evaluate the antidiabetic properties of the compounds. The Co (II) complex exhibited

the highest α-glucosidase inhibition, while the VO (IV) and Zn (II) complexes showed comparable efficiency against α-amylase. In ALP inhibition experiments, the HL ligand itself was inactive, but the complexes showed notable enzyme inhibition in a concentration-dependent manner, with the following order of effectiveness: VO (IV) > Zn (II) > Co(II) [36].

In the study, it was observed that the ligand HL bound to a specific pocket in α-glucosidase, distinct from the binding sites of the metal complexes. The binding site for HL was characterized by specific residues, including TYR1282, ASP1288, PHE1289, THR1290, LEU1291, PRO1299, TYR1328, PRO1329, ALA1330, ARG1333, GLU1397, GLU1400, LEU1401, ASN1404, PRO1405, GLN1406, ARG1410, SER1411, and LEU1412. In contrast, all metal complexes shared a common binding site composed of different residues. Notably, the Co (II) and VO(IV) complexes exhibited the highest binding energies and dissociation constants, indicating strong interactions with the enzyme. These findings suggest the potential of both metal complexes and ligands in inhibiting α-amylase and α-glucosidase, thus holding promise for antidiabetic therapy (Figure 2.6) [36].

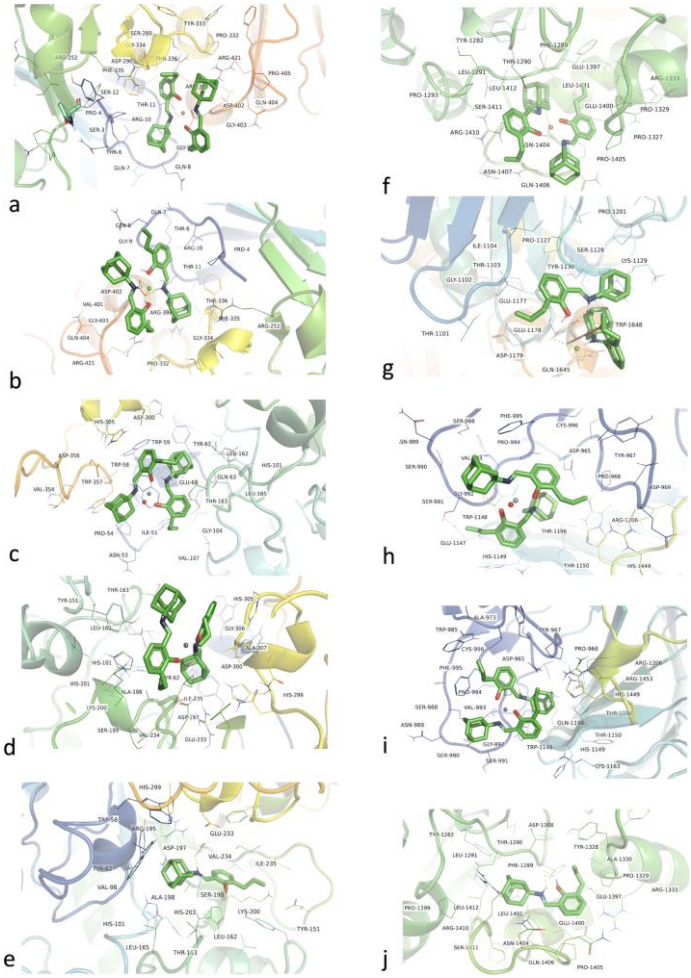


Figure 2.6. "α-Amylase" (a–e) and "α-glucosidase" (f–j) functionalities of the ligand and metal complexes [36].

According to the numerous investigations of the interaction between the bipyridine and metal complex that effects on the human body, current research is ongoing to demonstrate how these synthesized compounds affect digestive enzymes and cholinesterase. Some metal complexes have many uses, such as in the food and beverage industry as an acidity regulator, and some complexes include pyridine ring (cyclic nitrogen) that has important role as anticancer activity that reduces tumor size, and it is some of them a tetrahedral structure that has antimicrobial activity against some human pathogenic bacteria [37]. As a result, the research will may play an important role in this study since the applications of enzymes have risen due to improvements in industrial microbiology and biochemical engineering. The usage of enzymes such as amylase has grown into many new domains such as clinical,

pharmaceutical, and analytical chemistry. Because of the interaction of amylase's activity with alpha-glucose, it will be utilized on an inhibitory basis.

SECTION 3
MATERIAL AND METHOD

3.1. Chemicals

All chemicals and solvents used in this study were of high purity and used without further purification. The enzymes were obtained from Sigma Company. Other chemicals used in this study were phosphate buffer (Merc), sodium chloride, 4-nitrophenyl-D-glucopyranoside (Sigma Aldrich) , tris (hydroxymethyl) aminomethane (Sigma Aldrich) , acetonitrile, methanol (Sigma-Aldrich UN1230), starch solution, sodium hydroxide (Carlo Erba) , 3,5 dinitrosalicylic acid, and potassium sodium tartrate (Merc). Dr. İsmail YILMAZ provided us the metal complexes.

The metal complexes whose inhibitory effects on enzymes were examined in the study and the ligand structures from which these complexes were synthesized are given below.

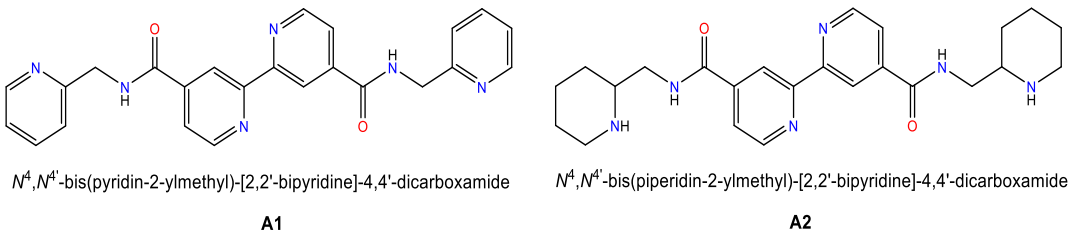


Figure 3.1. A1 and A2 ligand structures from which metal complexes are synthesized.

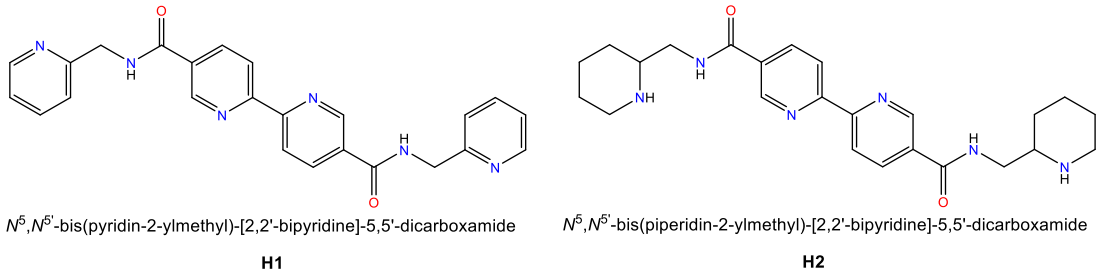


Figure 3.2. H1 and H2 ligand structures from which metal complexes are synthesized.

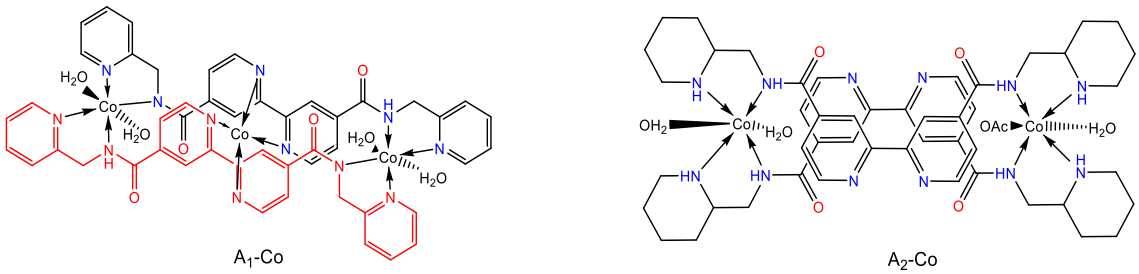


Figure 3.3. Predicted structure of the complexes of cobalt metal with A_1 and A_2 ligands.

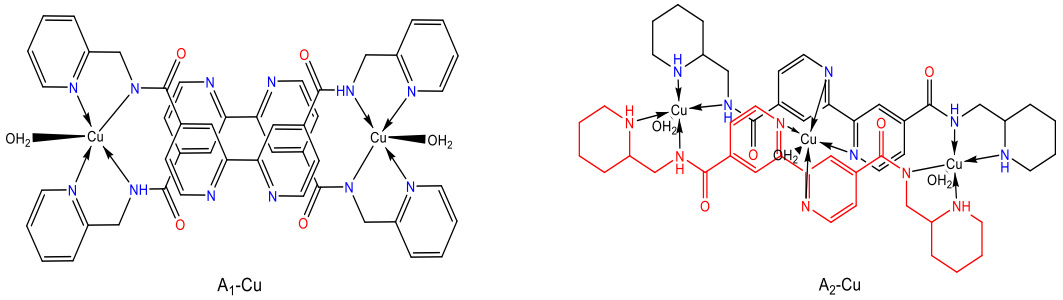


Figure 3.4. Predicted structure of the complexes of copper metal with A_1 and A_2 ligands.

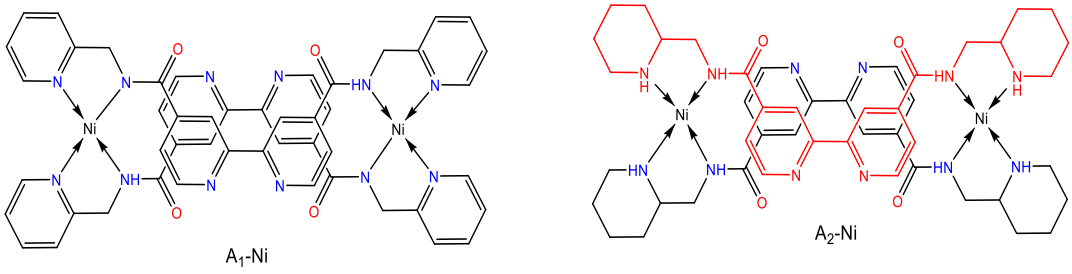
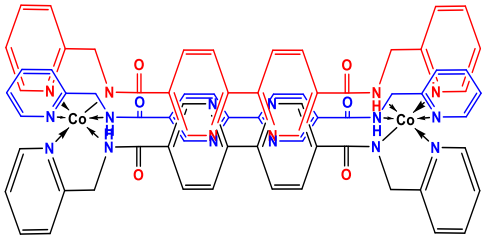
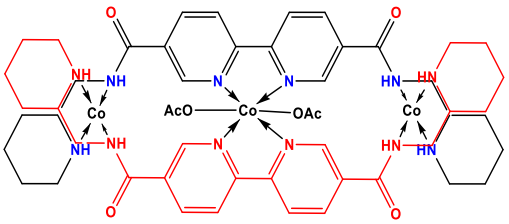


Figure 3.5. Predicted structure of the complexes of nickel metal with A_1 and A_2 ligands.

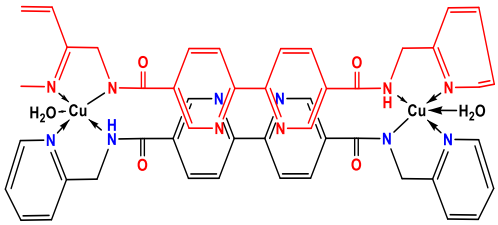


H_1-Co

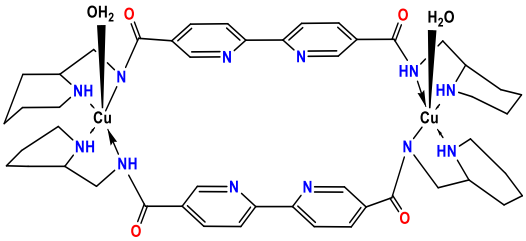


H_2-Co

Figure 3.6. Predicted structure of the complexes of cobalt metal with H_1 and H_2 ligands.

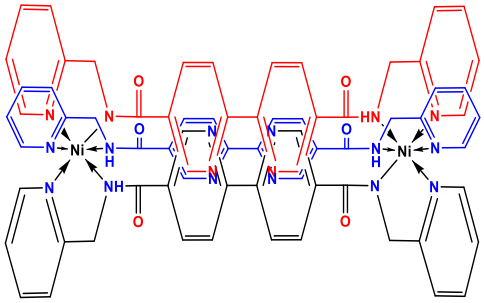


H_1-Cu

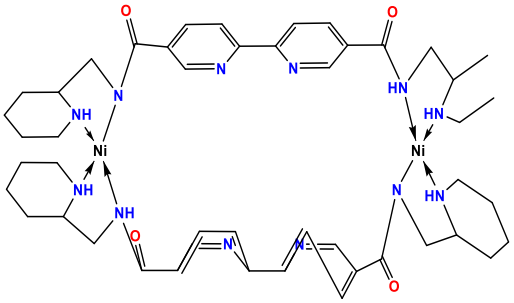


H_2-Cu

Figure 3.7. Predicted structure of the complexes of copper metal with H_1 and H_2 ligands.



H_1-Ni



H_2-Ni

Figure 3.8. Predicted structure of the complexes of nickel metal with H_1 and H_2 ligands.

3.2. Equipment Instrument

Vortex Shaker - Made by Wiggens.

Analytical Balances - Radwag

Bench Meter -- PH 50 Vio Lab - XS instruments

Magnetic stirrer plate lab – WF-H380a- weightlab instruments

Ultra sonic water bath cleaning - Mipr Lab

Mps series platelet shakers- Mipr Lab

Microplate reader - Thermo Scientific

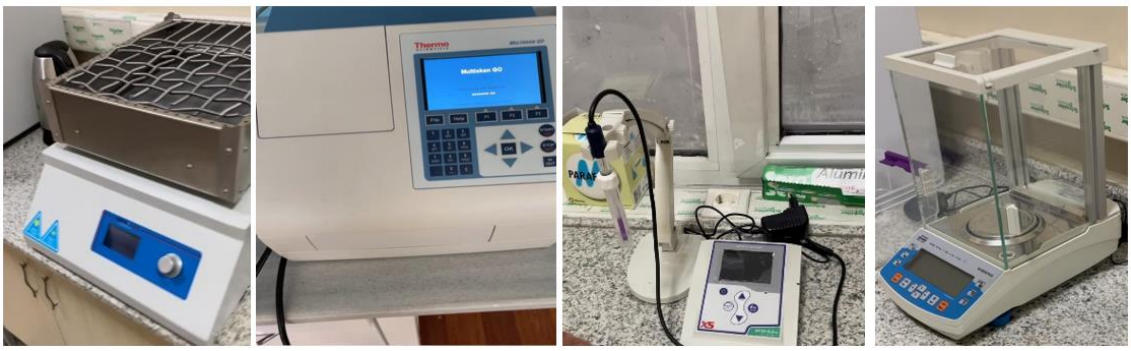


Figure 3.9. Instruments utilized.

3.3. Preparation Method

In the first stage, we used 1 mg/mL per tube, as well as 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.01 mg/mL, 0.005 mg/mL, 0.002 mg/mL of inhibitor. We used the same volume of varied concentration solutions.

Figure 3.10. Preparation of materials

3.1.1. Alpha Glucosidase Assay

We prepared by pipette 100 μL from 100 mM Phosphate Buffer pH 6.8, with 40 μL from substrate 4-Nitrophenyl-D-Glucopyranoside (5 mM prepare 7.4 mg 5 ml dissolved in water), we used enzyme with 40 μL.

3.1.2. Pancreatic Lipase Assay

Lipase was produced by dissolving 10 mg/ml in Buffer and centrifuging it. And in pipette 50 μL, we added 20 mM Tris + 20 mM NaCl with pH 8.
1 mg pnfd is dissolved in 333 μL Acetonitrile for pnfdekonat. Slowly dissolve 150 μL of it and 400 μL of methanol in 3.5 ml of Buffer. It was leave a suitable period between measurements. Then we use enzyme in 50 μL and distilled water by 80 μL.

3.1.3. Acetylcholinesterase Assay

Using a microplate, we made Acetylcholinesterase with 125 μL DTNB and 25 μL of enzyme substrate (Acetylcholine Iodine), 50 μL Tris, pH 8, and 25 μL inhibitor solutions. For the control, there is no inhibitor and enzyme at blank 2, and there is no inhibitor at blank 3 and 4.

3.1.3. Butyrylcholinesterase Assay

Using a microplate, we made Butyrylcholinesterase with 125 μL DTNB and 25 μL of enzyme substrate, 50 μL Tris, pH 8, and 25 μL inhibitor solutions. For the control, there is no inhibitor and enzyme at blank 2, and there is no inhibitor at blank 3 and 4.

3.4. Docking Studies

Molecular docking provides insight into the points and types of interactions between ligands and receptors. This process involves placing one or more ligands on the high-resolution structure of a receptor and determining the potential interaction points of these ligands with the receptor. Docking algorithms can predict ligand-protein complex geometries, ligand binding affinity, and protein and ligand pose that may result from interaction. Maestro 12.5 program of Schrödinger Molecular Modeling Suite was used in the study. The software uses two important programs when performing operations. These are Emodel and GlideScore. EmodelScore EmodelScorese determines the ideal posture of the ligand. GlideScore gives ligand binding affinity results. Three-dimensional crystal structures of proteins were obtained from the RCSB Protein Data Bank. In this study, the structures specified with PDB codes 4TVK for AChE enzyme, 4BDS for BChE, and 1LPB for pancreatic lipase were transferred to Maestro Release 2023-1. Protein preparation for the docking study was performed using Schrödinger's Protein Preparation Wizard. Identification of the active site of the prepared enzyme was performed with Maestro's Receptor Grid Generation Application. The molecular structures of the ligands used in the synthesis of metal complexes were drawn in the ChemDraw 18.0 application, and the molecular structures of the standard inhibitors of the enzymes were taken from the www.pubchem.ncbi.nlm.nih.gov page and uploaded to the program. These molecules were made ready for docking with Maestro's Ligprep application. Glide/XP was used to dock all compounds into the enzymes mentioned above. The conformations of the ligands with the lowest binding free energy were evaluated. Interaction diagrams and connection patterns were obtained with Maestro Release 2023-1.

SECTION 4

INVESTIGATION RESULTS

4.1. The Standard Graphic Used for Quantitative of Enzymes Inhibition

The effect of metal complex on the enzymes were determined using the *in vitro* method. Accordingly, activity measurements were made for each enzyme at least five different complex concentrations. Control measurements were assumed to be 100% and activity values were calculated for different concentrations. Using these values, graphs of complex concentration versus % activity were drawn.

4.1.1. Examining the Impact of Metal Complexes on α-Glucosidase Activity

In this study, the effect of metals complex on alpha glucosidase enzyme was examined. As a result of the measurements made at 408 nm, the concentrations of metal complexes were plotted against the % activity.

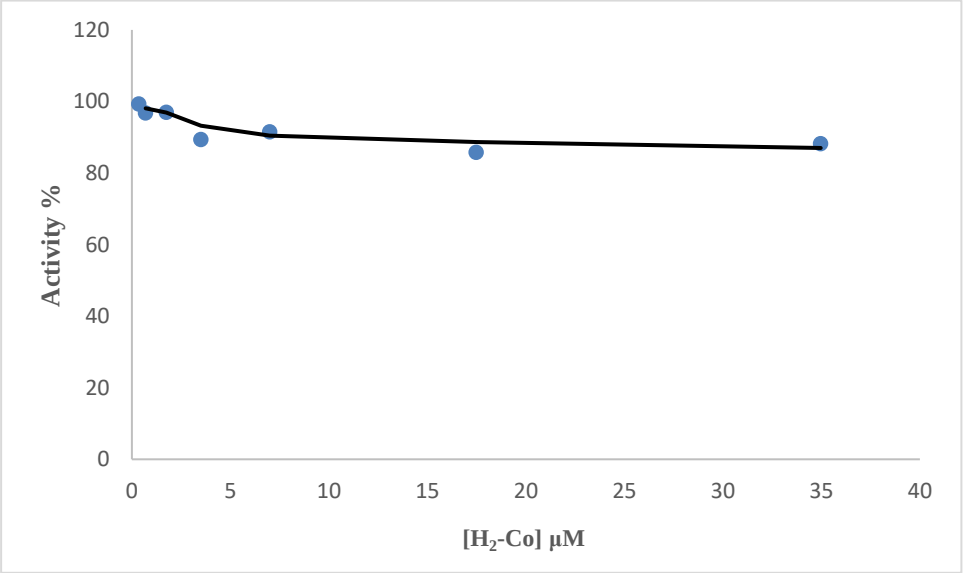


Figure 4.1. Impact of H_2-Co complex on α-glycosidase enzyme activity.

The effect of (H_2-Co) complex on α-glycosidase enzyme was examined in the range of 0.35-35 µM concentrations. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.1).

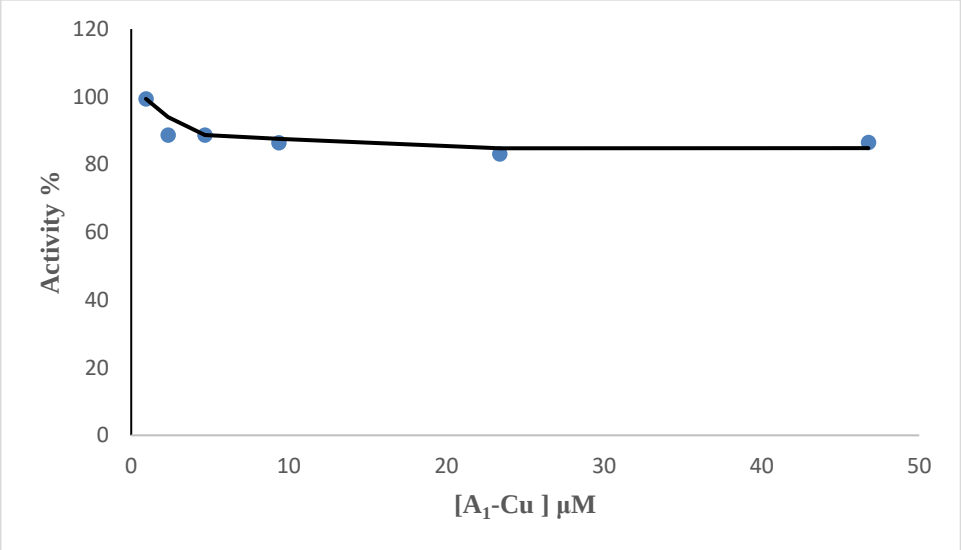


Figure 4.2. A_1-Cu complex effect on α-glycosidase.

The effect of (A_1-Cu) metal complex on α-glycosidase enzyme was investigated at concentrations ranging from 0.93-46.8 µM. According to the results, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.2).

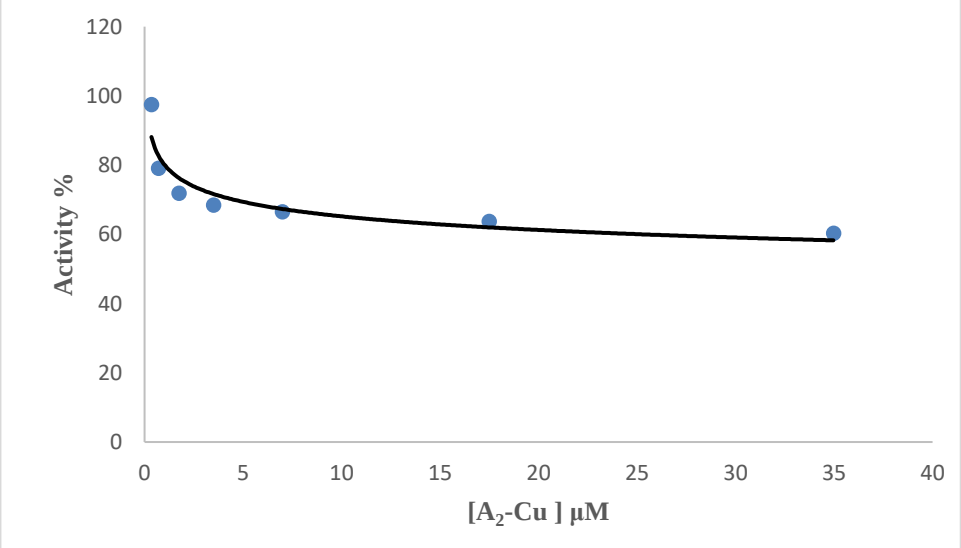


Figure 4.3. Influence of A_2-Cu complex on α-glycosidase enzyme function.

The effect of (A_2-Cu) complex was examined in the concentration range of 0.35-35 µM. According to the results obtained, it was found that the complex reduced the activity to 60.35% in the concentration range studied (Figure 4.3).

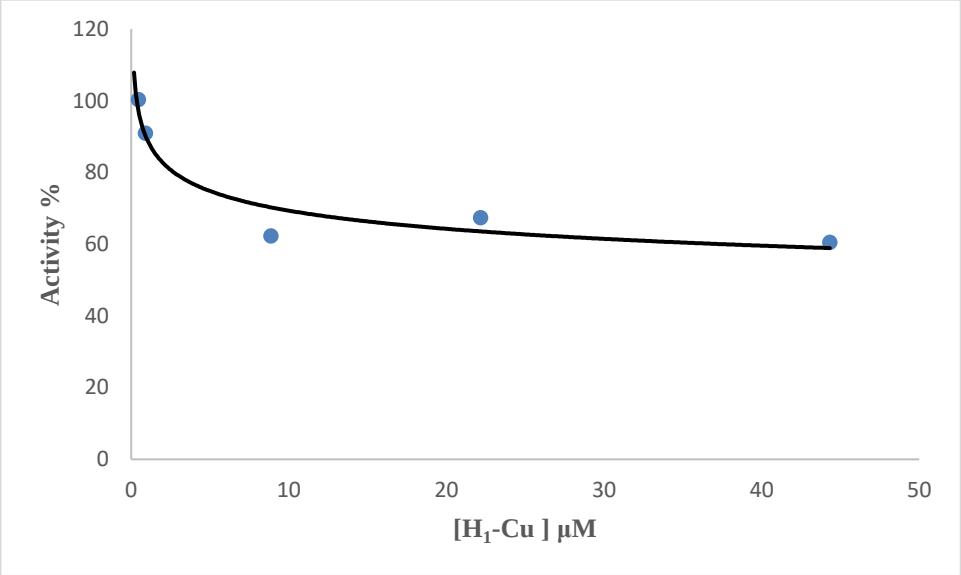


Figure 4.4. Impact of H_1-Cu complex on α-glycosidase enzyme function.

The effect of (H_1-Cu) complex was examined in the concentration range of 0.44- 44.32 µM. According to the results obtained, it was found that the complex reduced the activity to 60.55% in the concentration range studied (Figure 4.4).

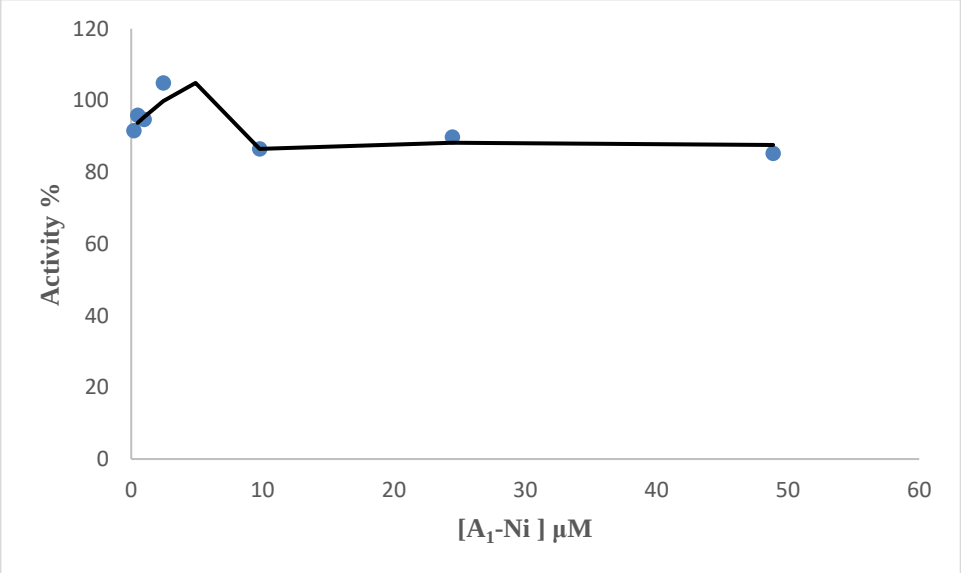


Figure 4.5. Impact of A_1-Ni complex on α-glycosidase enzyme function.

The effect of (A_1-Ni) complex was examined in the concentration range of 0.196-48.88 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.5).

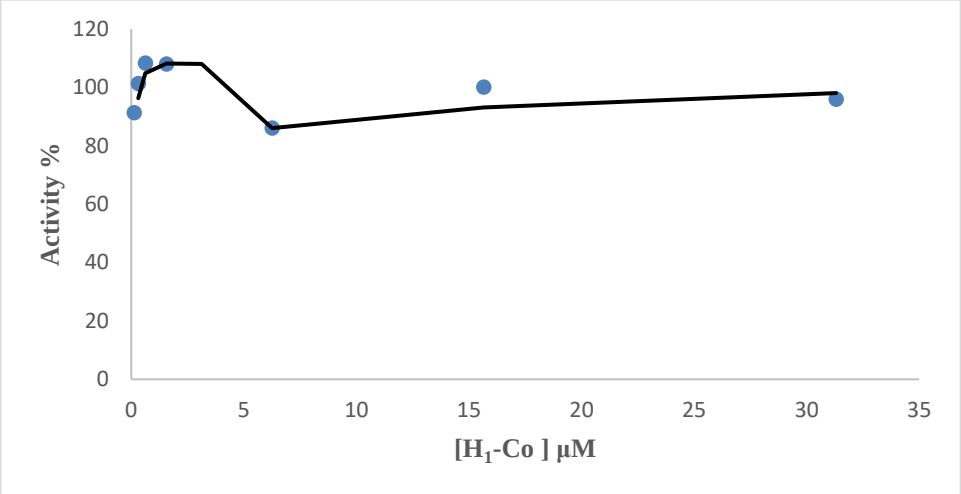


Figure 4.6. Impact of H_1-Co complex on α-glycosidase enzyme function.

The effect of (H_1-Co) complex was examined in the concentration range of 0.125-31.31 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.6).

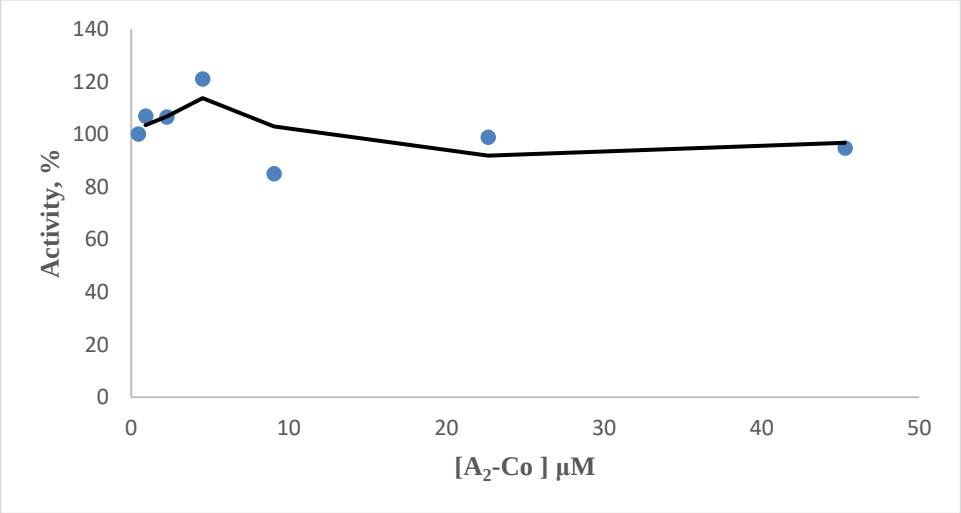


Figure 4.7. Impact of A_2-Co complex on α-glycosidase enzyme function.

The effect of (A_2-Co) complex was examined in the concentration range of 0.453-45.29 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.7).

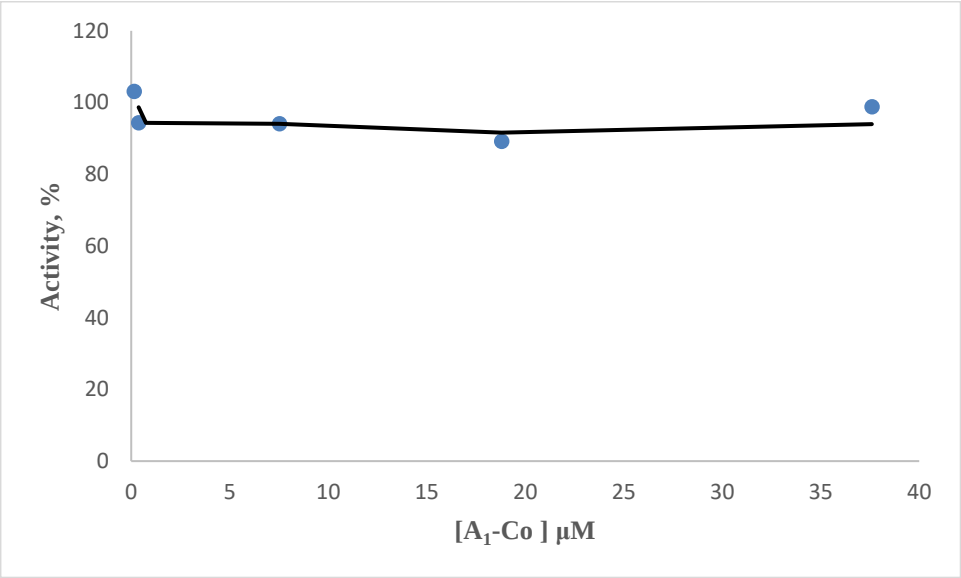


Figure 4.8. Impact of A_1-Co complex on α-glycosidase enzyme function.

The effect of (A_1-Co) complex was examined in the concentration range of 0.15-37.6 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.8).

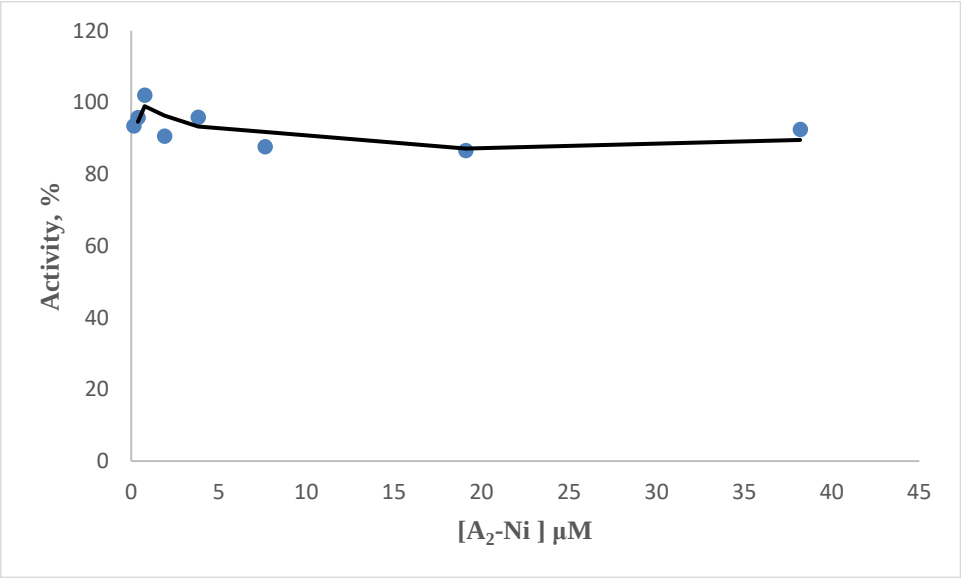


Figure 4.9. Impact of A_2-Ni complex on α-glycosidase enzyme function.

The effect of (A_2-Ni) complex was examined in the concentration range of 0.15-38.2 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.9).

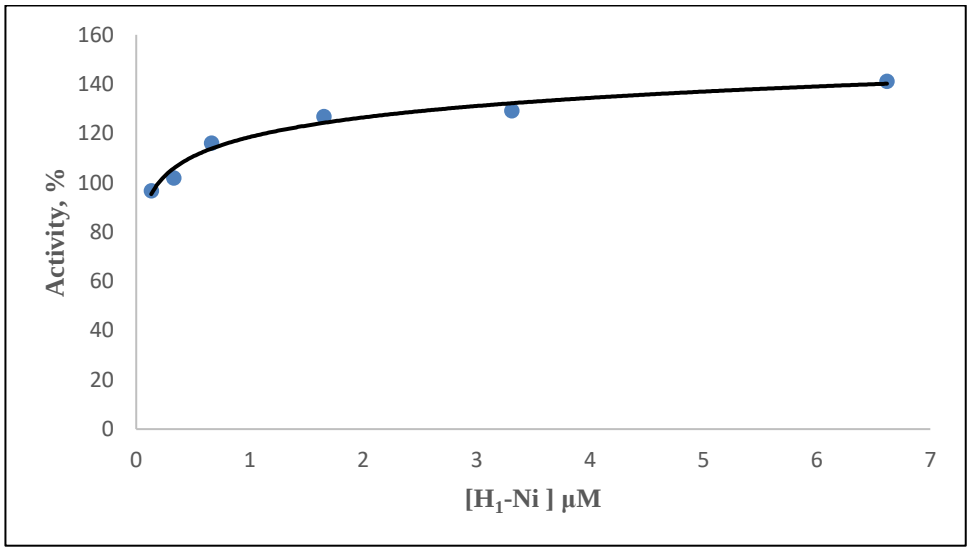


Figure 4.10. H_1-Ni complex modulation of α-glycosidase enzyme activity.

The effect of (H_1-Ni) metal complex on α-glycosidase enzyme was investigated at concentrations ranging from 0.13-6.62 µM. According to the results, it was found that the complex activated the enzyme by 41% in the concentration range investigated (Figure 4.10).

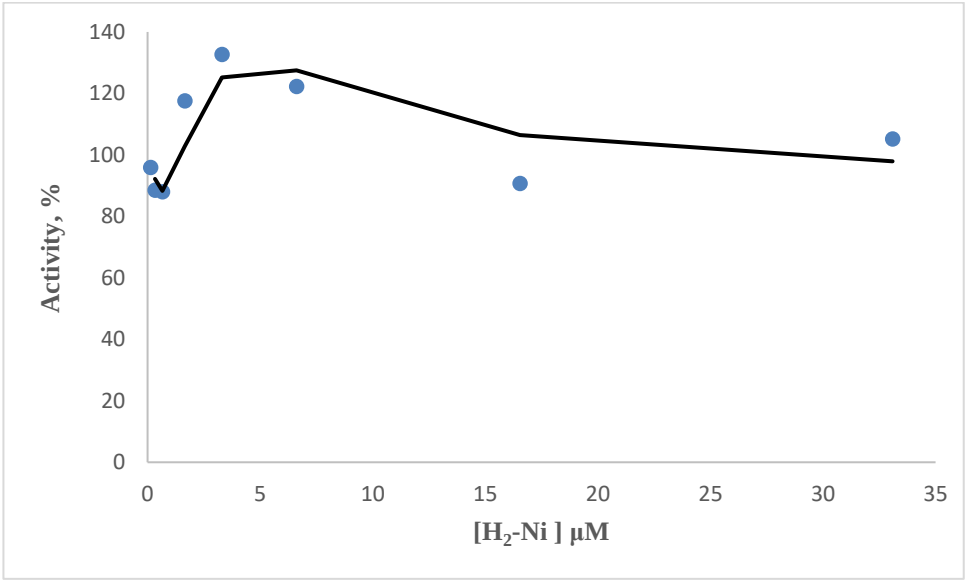


Figure 4.11. Effect of H_2-Ni complex on the activity of α-glycosidase enzyme.

The effect of (H_2-Ni) complex was examined in the concentration range of 0.13-33.1 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.11).

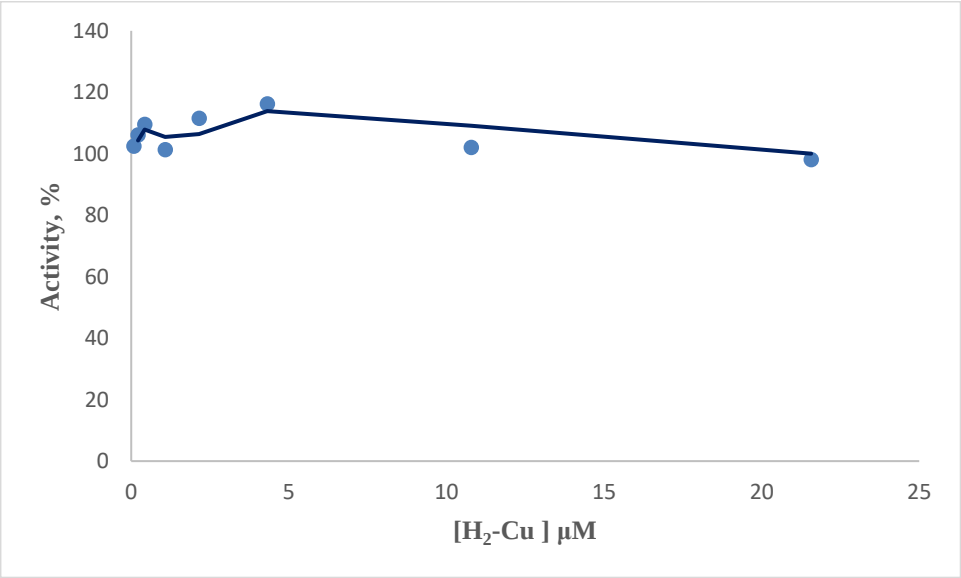


Figure 4.12. The influence of the H_2-Cu complex on α-glycosidase.

The effect of (H_2-Cu) complex was examined in the concentration range of 0.086-21.57 µM. According to the results obtained, it was found that the complex had no linear effect on the enzyme in the concentration range investigated (Figure 4.12).

4.1.2. Analyzing the Influence of Metal Complexes on Acetylcholinesterase (AChE) Activity

The effects of metal complexes on the AChE enzyme were examined by activity measurements at 405 nm. Measurements were made at least five different metal complex concentration and the results were evaluated by plotting graphs against relative activity percentage.

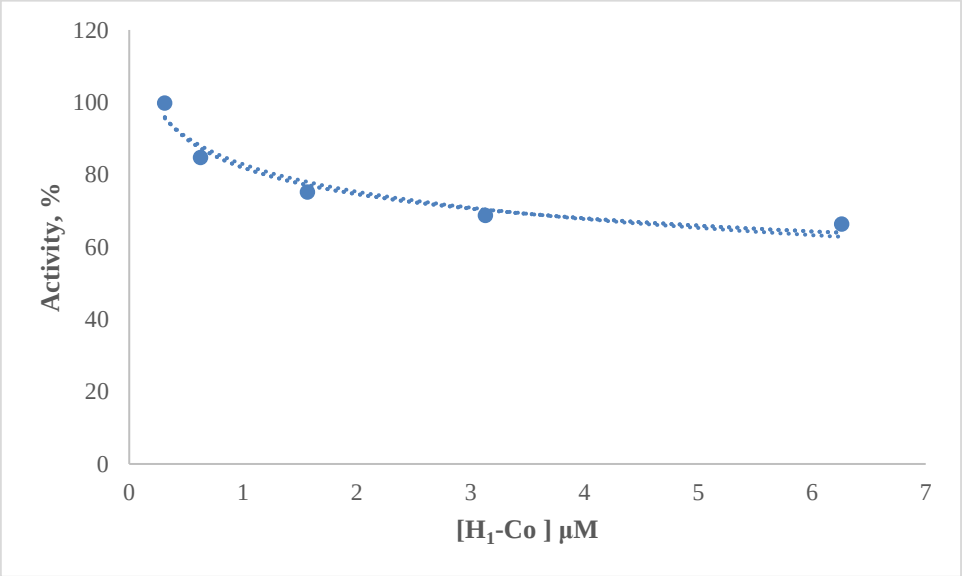


Figure 4.13. The impact of the H_1-Co complex on AChE.

The effect of (H_1-Co) complex on acetylcholinesterase enzyme activity was examined in the range of 0.31-6.26 µM concentrations. According to the results obtained, it was observed that the H_1-Co complex reduced the activity to 66.35% at a concentration of 6.26 µM. The IC_{50} value was not calculated since the activity could not be less than 50% (Figure 4.13).

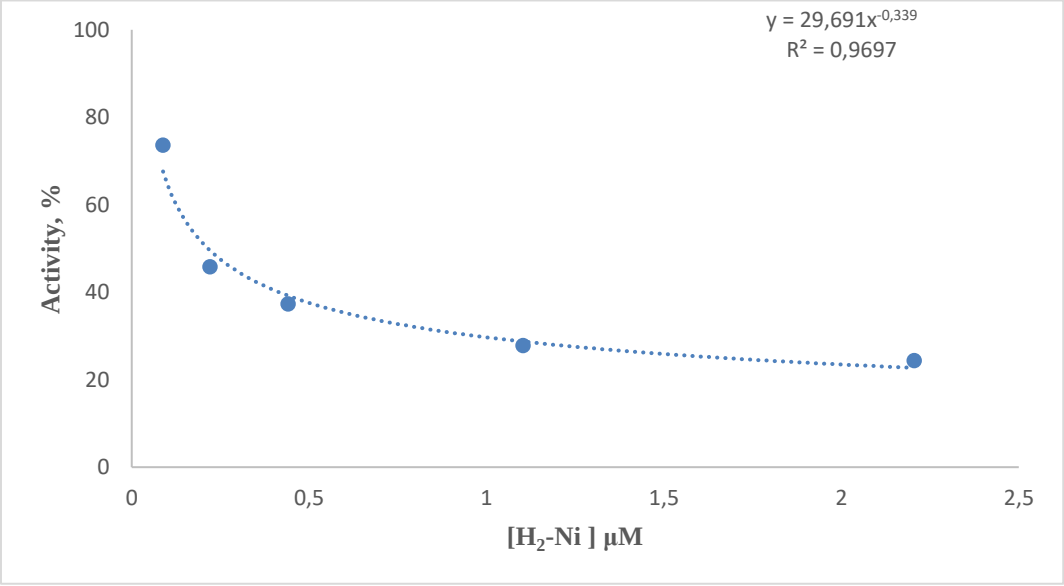


Figure 4.14. The influence of the H_2-Ni complex on AChE.

The effect of (H_2-Ni) complex on acetylcholinesterase enzyme activity was examined in the range of 0.088-2.20 µM concentrations. According to the results obtained, the

H_2-Ni complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 1.03 µM (Figure 4.14).

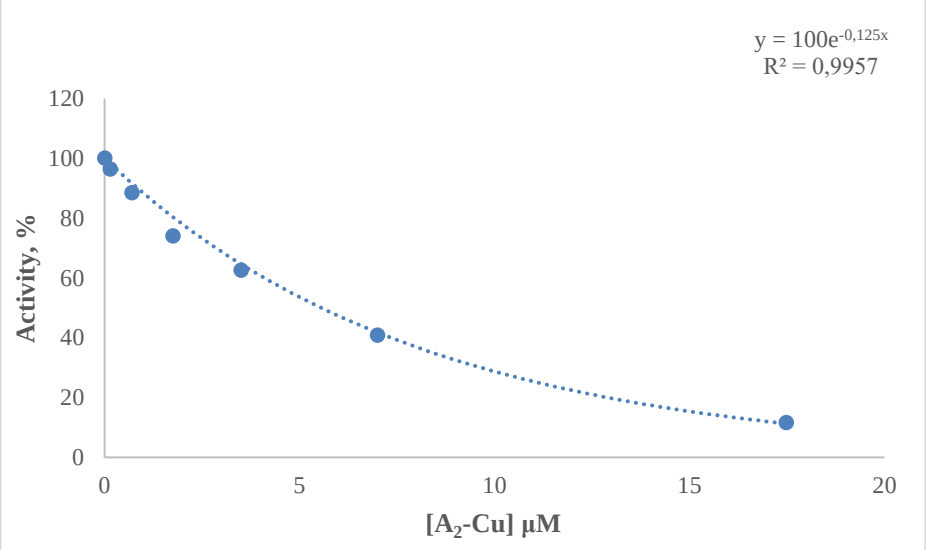


Figure 4.15. Impact of A_2-Cu complex on AChE.

The effect of (A_2-Cu) complex on acetylcholinesterase enzyme activity was examined in the range of 0.13-17.48 µM concentrations. According to the results obtained, the A_2-Cu complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 5.545 µM (Figure 4.15).

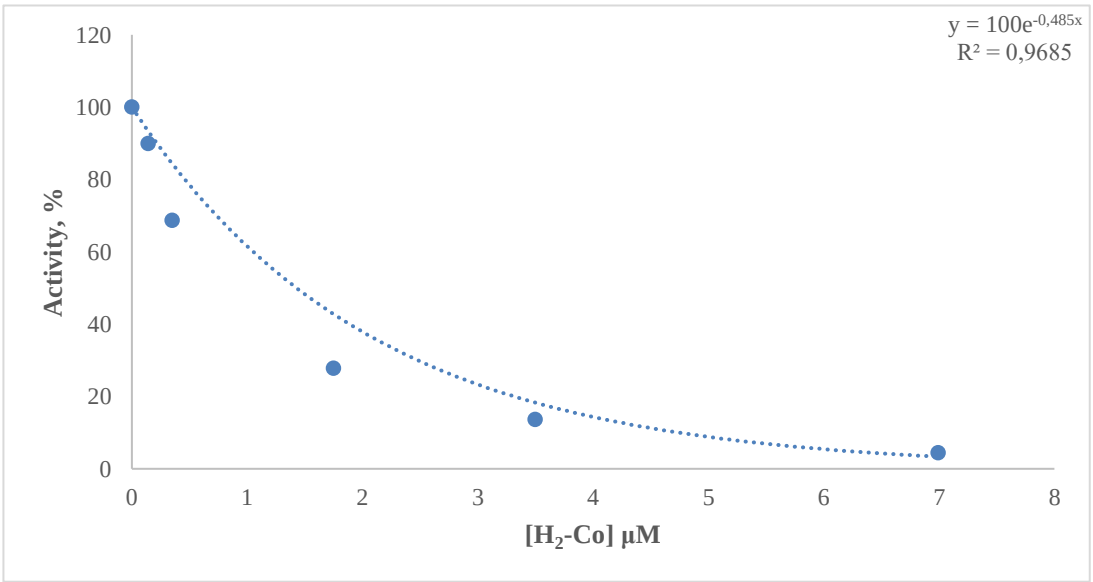


Figure 4.16. The effect of the H_2-Co complex on the functioning of the AChE.

The effect of (H_2-Co) complex on acetylcholinesterase enzyme activity was examined in the range of 0.14-6.99 µM concentrations. According to the results obtained, the H_2-Co complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 1.43 µM (Figure 4.16).

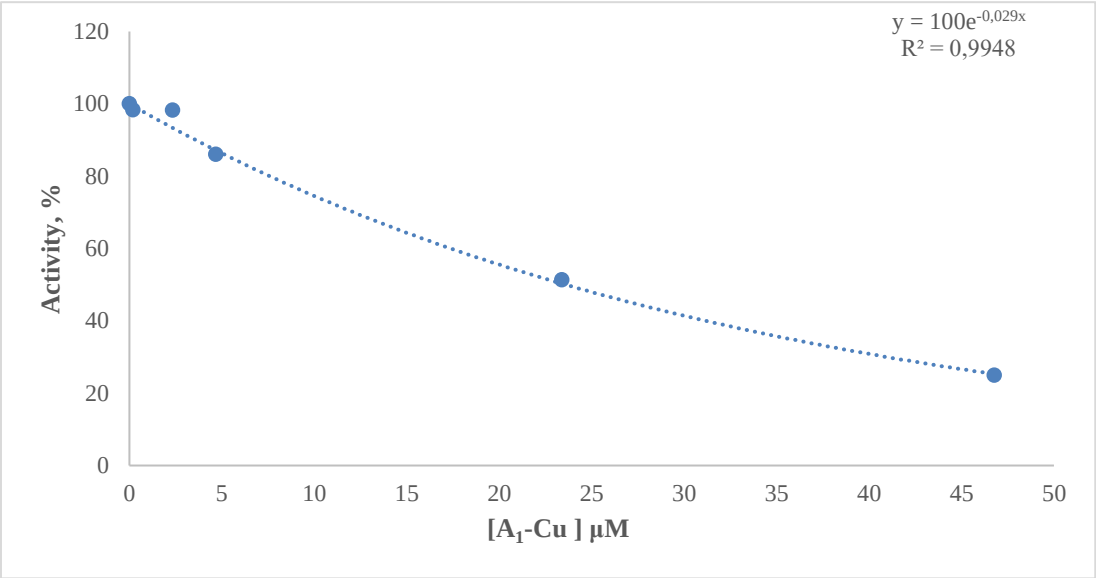


Figure 4.17. Influence of A_1-Cu complex on AChE enzyme function.

The effect of (A_1-Cu) complex on acetylcholinesterase enzyme activity was examined in the range of 0.19-46.8 µM concentrations. According to the results obtained, the A_1-Cu complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 23.9 µM (Figure 4.17).

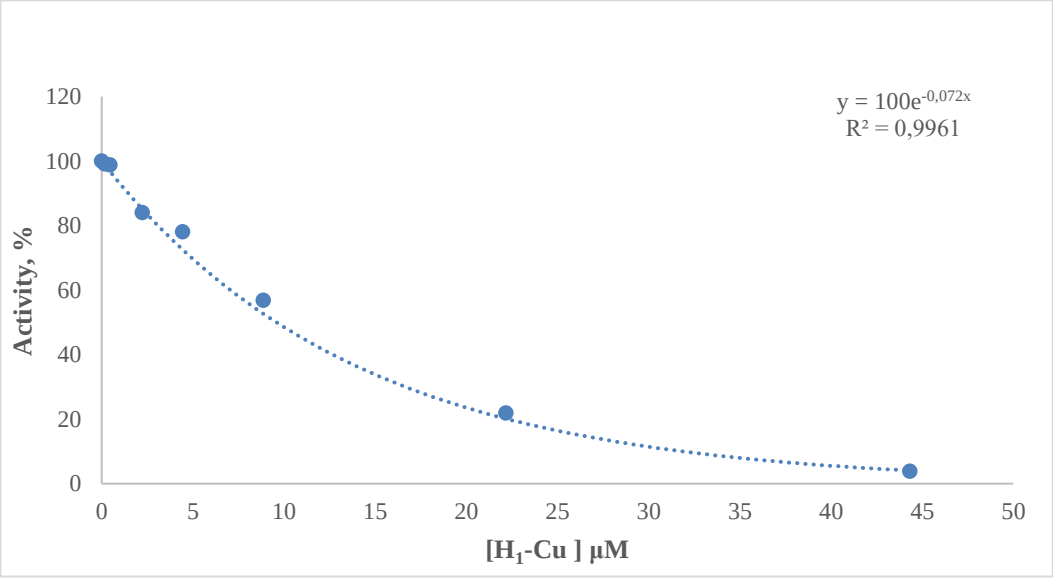


Figure 4.18. The effect of H_1-Cu complex on AChE.

The effect of (H_1-Cu) complex on acetylcholinesterase enzyme activity was examined in the range of 0.18-44.32 µM concentrations. According to the results obtained, the H_1-Cu complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 9.63 µM (Figure 4.18).

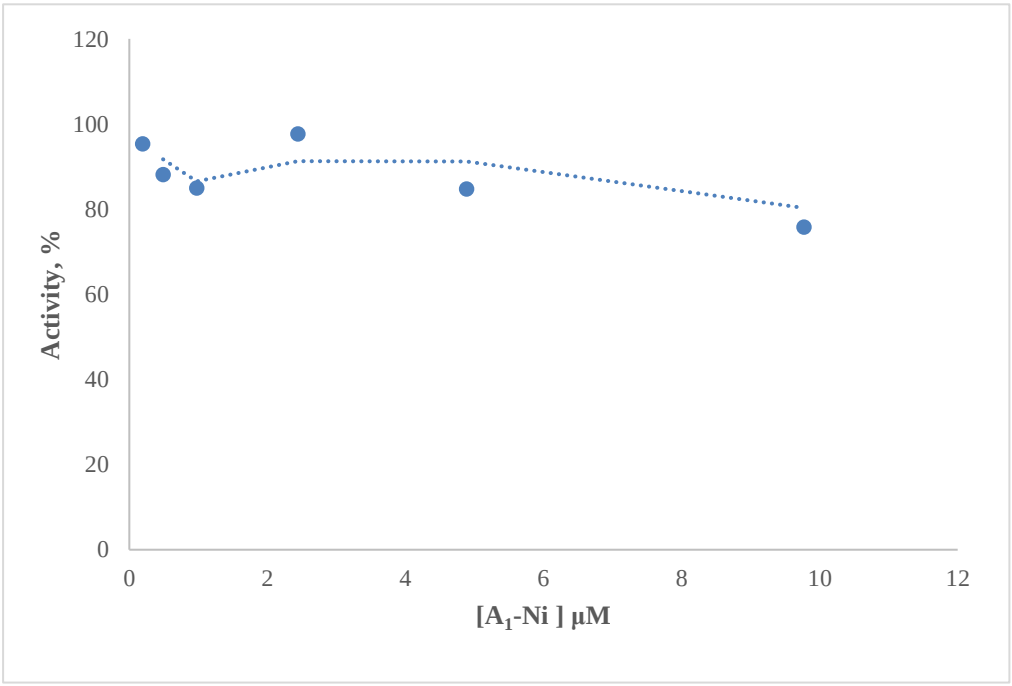


Figure 4.19. The effect of A_1-Ni complex on AChE.

The effect of (A_1- Ni) complex was examined in the concentration range of 0.19-9.8 µM. According to the results obtained, although a partial activation was observed at certain concentrations, a linear effect was not observed (Figure 4.19).

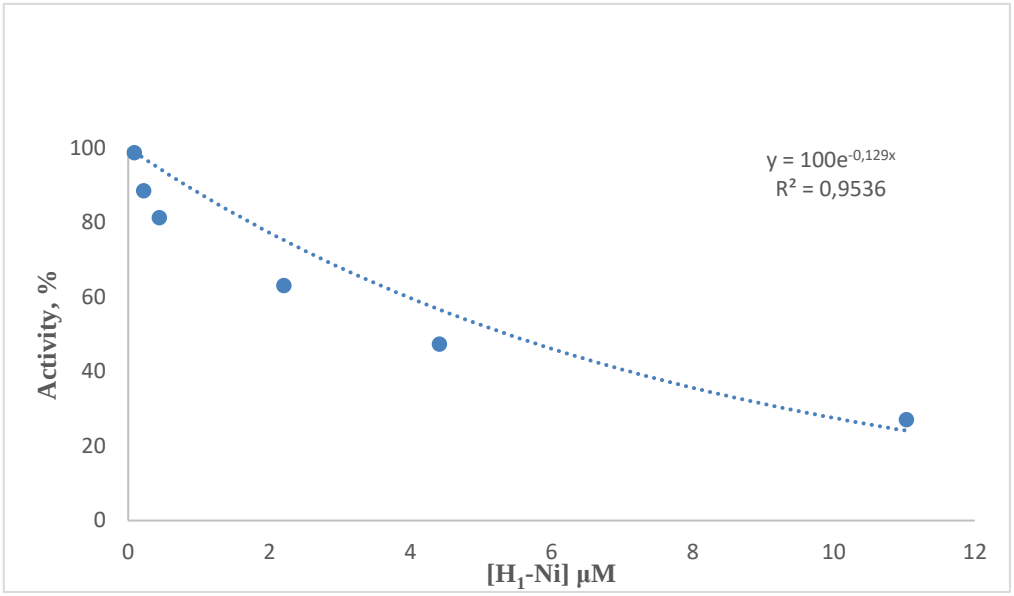


Figure 4.20. The influence of the H_1-Ni complex on the enzymatic activity of AChE enzyme.

The effect of (H_1-Ni) complex on acetylcholinesterase enzyme activity was examined in the range of 0.088-11.03 µM concentrations. According to the results obtained, the H_1-Ni complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 5.373 µM (Figure 4.20).

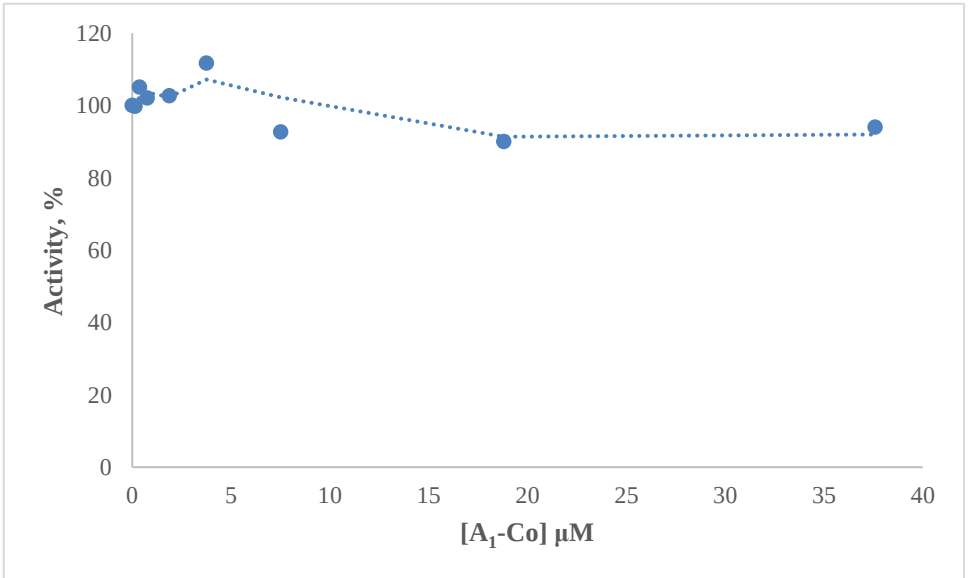


Figure 4.21. Effect of A_1-Co complex on the activity of AChE.

The effect of (A_1- Co) complex was examined in the concentration range of 0.15-37.60 µM. According to the results obtained a linear effect was not observed (Figure 4.21).

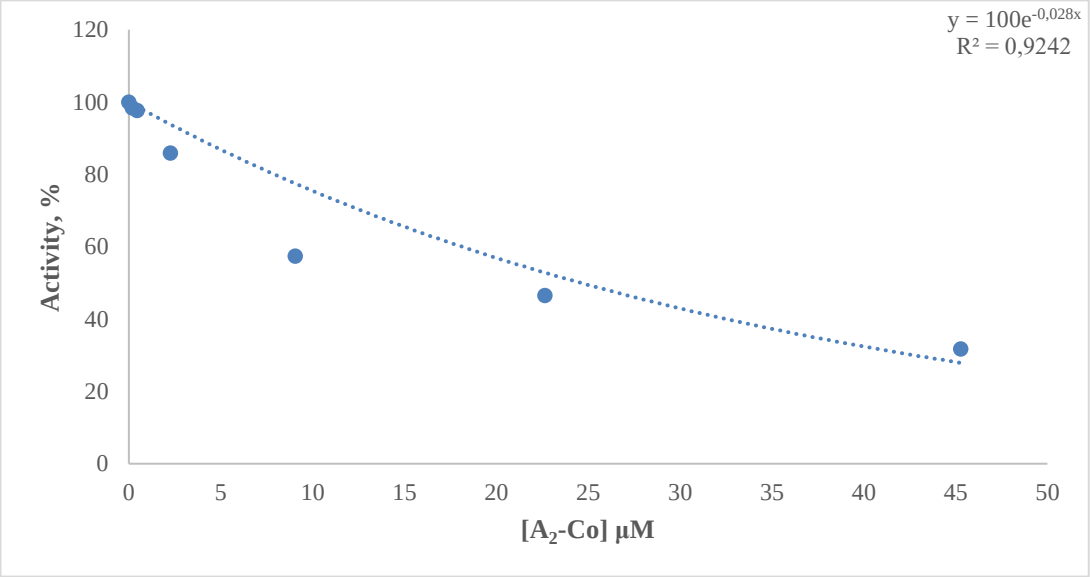


Figure 4.22. A_2-Co complex effects AChE activity.

The effect of (A_2-Co) complex on acetylcholinesterase enzyme activity was examined in the range of 0.18-45.29 µM concentrations. According to the results obtained, the (A_2-Co) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 24.76 µM (Figure 4.22).

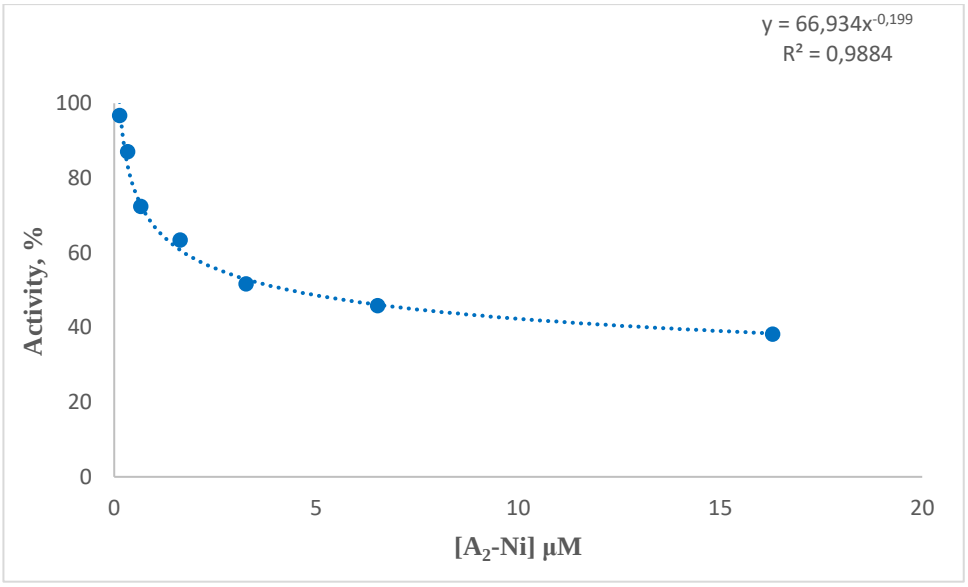


Figure 4.23. A_2-Ni complex effects AChE activity.

The effect of (A_2-Ni) complex on acetylcholinesterase enzyme activity was examined in the range of 0.36-0.14 µM concentrations. According to the results obtained, the (A_2-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 4.33 µM (Figure 4.23).

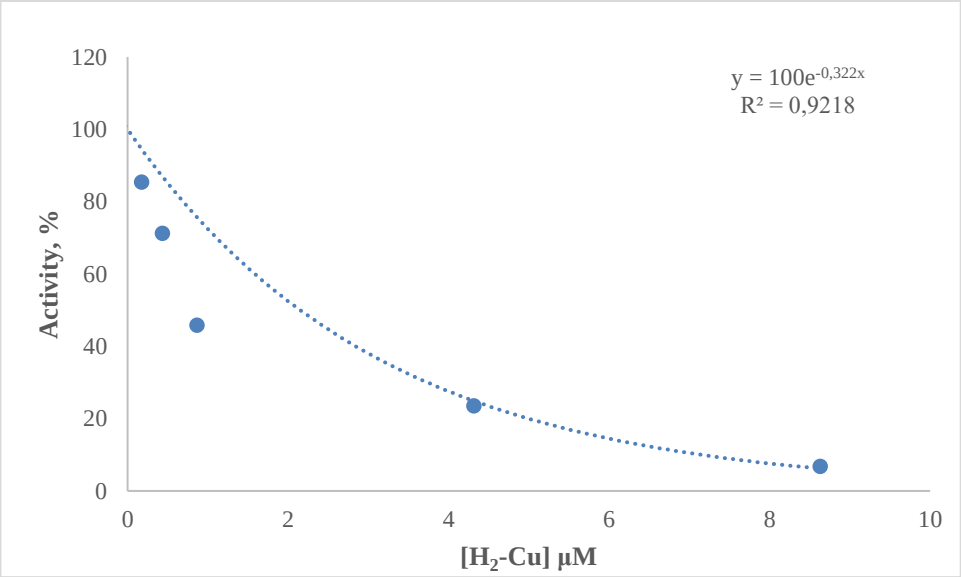


Figure 4.24. Effect of H_2-Cu complex on the activity of AChE.

The effect of (H_2-Cu) complex on acetylcholinesterase enzyme activity was examined in the range of 0.17-8.63 µM concentrations. According to the results obtained, the H_2-Cu complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 2.15 µM (Figure 4.24).

4.1.3. The Effect of Metal Complexes on Butyrylcholinesterase (BChE) Activity

The effects of metal complexes on the BChE enzyme were examined by activity measurements at 405 nm. Measurements were made at least five different metal complex concentrations and the results were evaluated by plotting graphs against relative activity percentage.

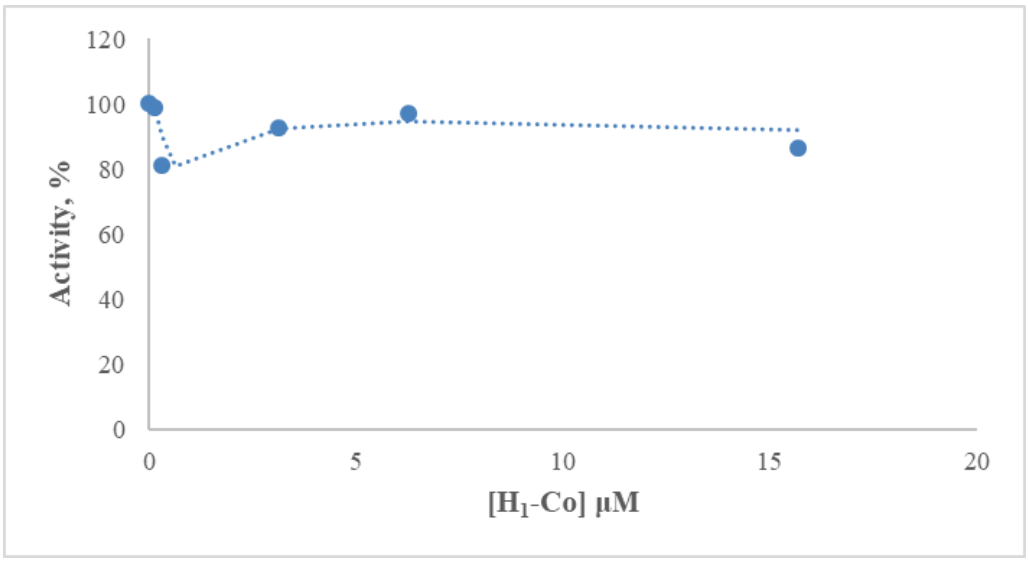


Figure 4.25. Impact of H_1-Co complex on BChE activity.

The effect of (H_1- Co) complex was examined in the concentration range of 0.13-15.7 µM. According to the results obtained, a linear effect was not observed (Figure 4.25).

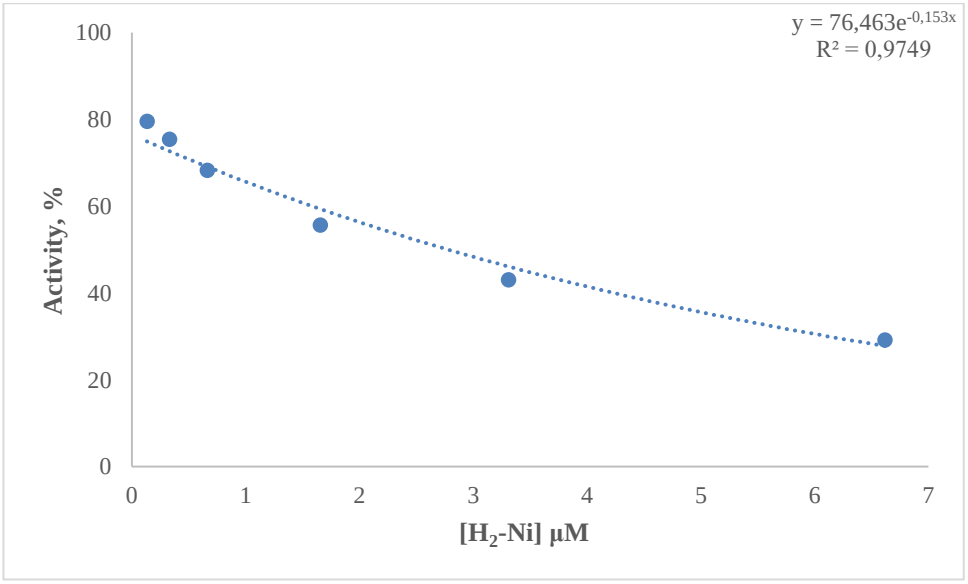


Figure 4.26. H_2-Ni effect on BChE.

The effect of (H_2-Ni) complex on butyrylcholinesterase enzyme activity was examined in the range of 0.13-6.62 µM concentrations. According to the results obtained, the (H_2-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 4.53 µM (Figure 4.26).

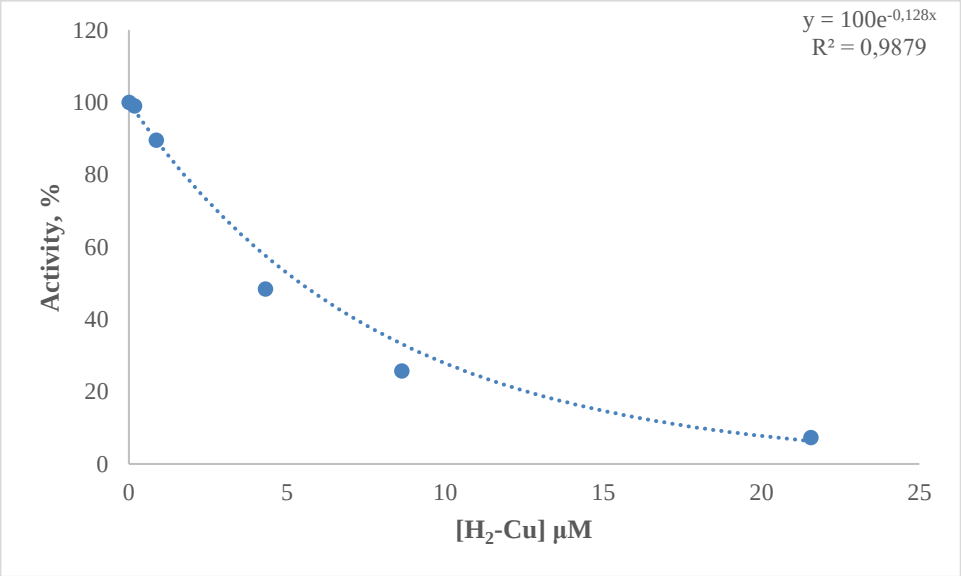


Figure 4.27. Influence of H_2-Cu complex on BChE.

The effect of (H_2-Cu) complex on BChE enzyme activity was examined in the range of 0.17-21.6 µM concentrations. According to the results obtained, the (H_2-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 5.42 µM (Figure 4.27).

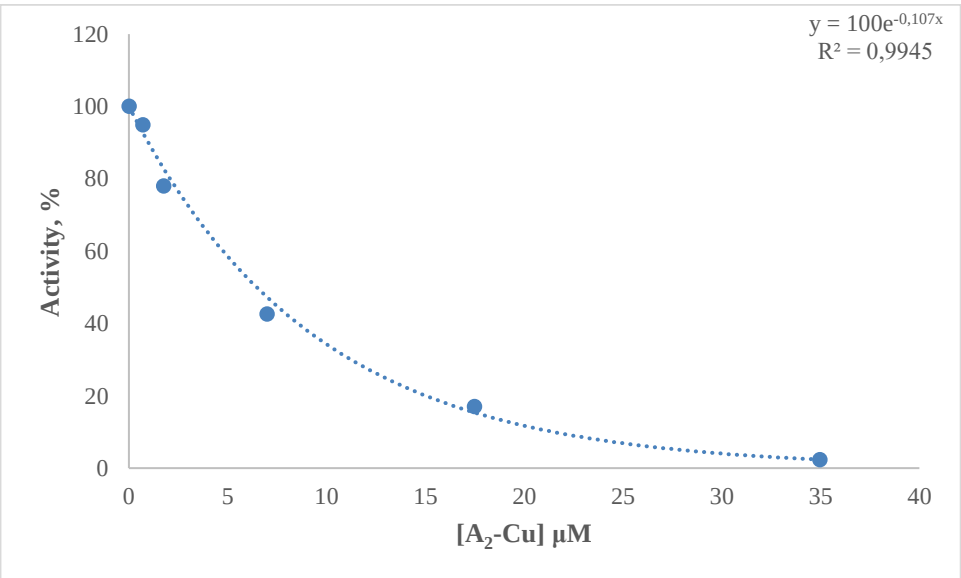


Figure 4.28. The effect of A_2-Cu complex on BChE.

The effect of (A_2-Cu) complex on BChE enzyme activity was examined in the range of 0.7-34.96 µM concentrations. According to the results obtained, the (A_2-Cu)

complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 6.48 µM (Figure 4.28).

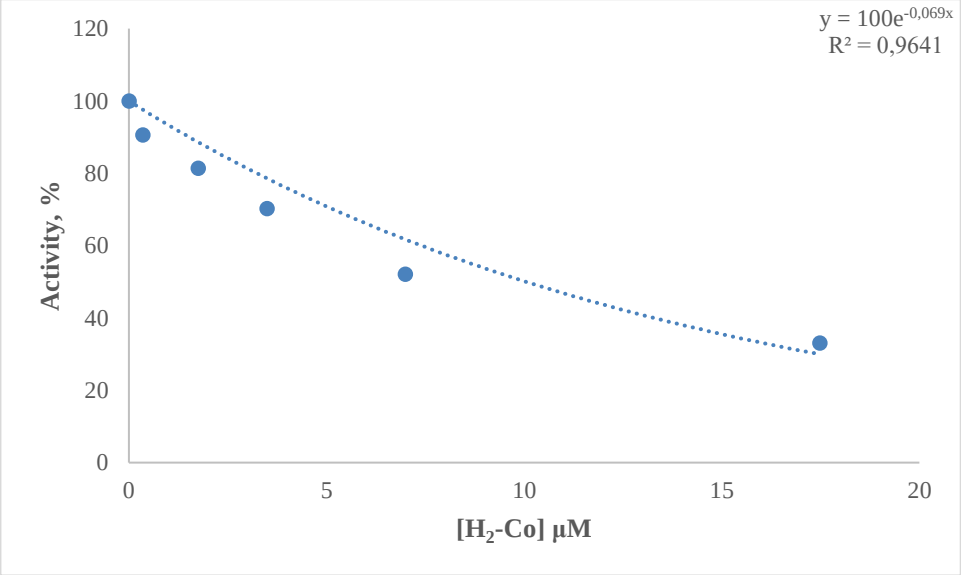


Figure 4.29. H_2-Co complex modulation of BChE.

The effect of (H_2-Co) complex on BChE enzyme activity was examined in the range of 0.35-17.48 µM concentrations. According to the results obtained, the (H_2-Co) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 10.05 µM (Figure 4.29).

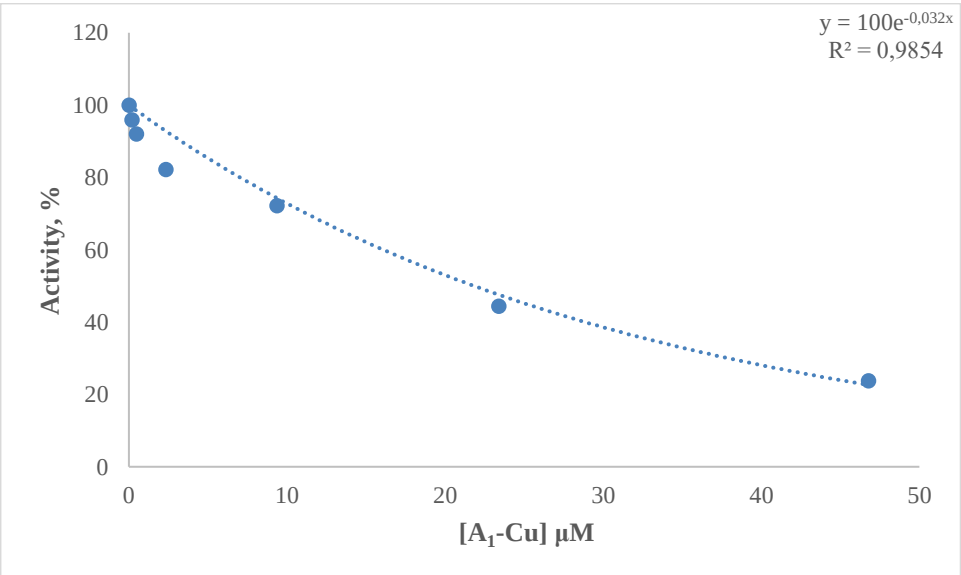


Figure 4.30. The effect of A_1-Cu complex on BChE.

The effect of (A_1-Cu) complex on BChE enzyme activity was examined in the range of 0.19-49.77 µM concentrations. According to the results obtained, the (A_1-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 21.66 µM (Figure 4.30).

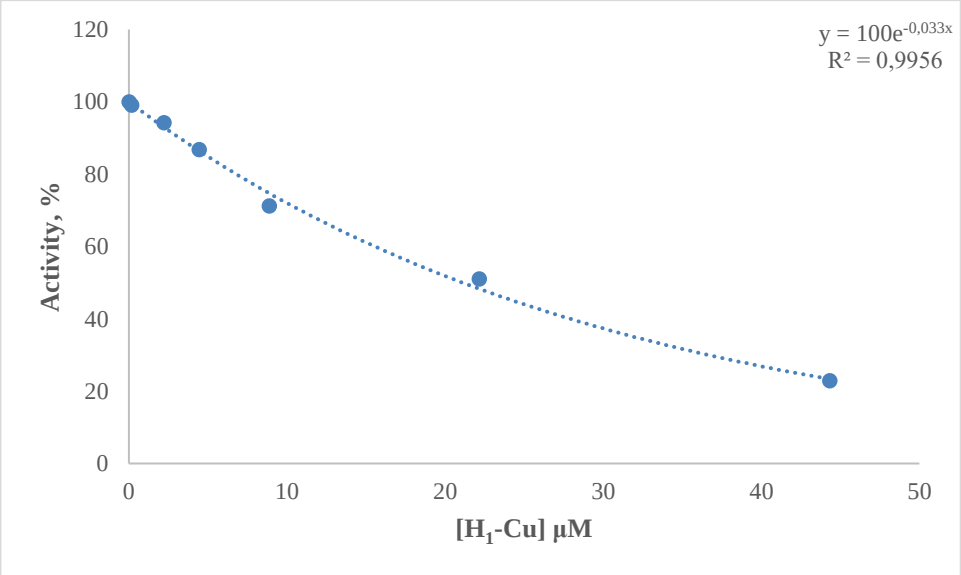


Figure 4.31. H_1-Cu complex modulation of BChE activity.

The effect of (H_1-Cu) complex on BChE enzyme activity was examined in the range of 0.18-44.33 µM concentrations. According to the results obtained, the (H_1-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 21 µM (Figure 4.31).

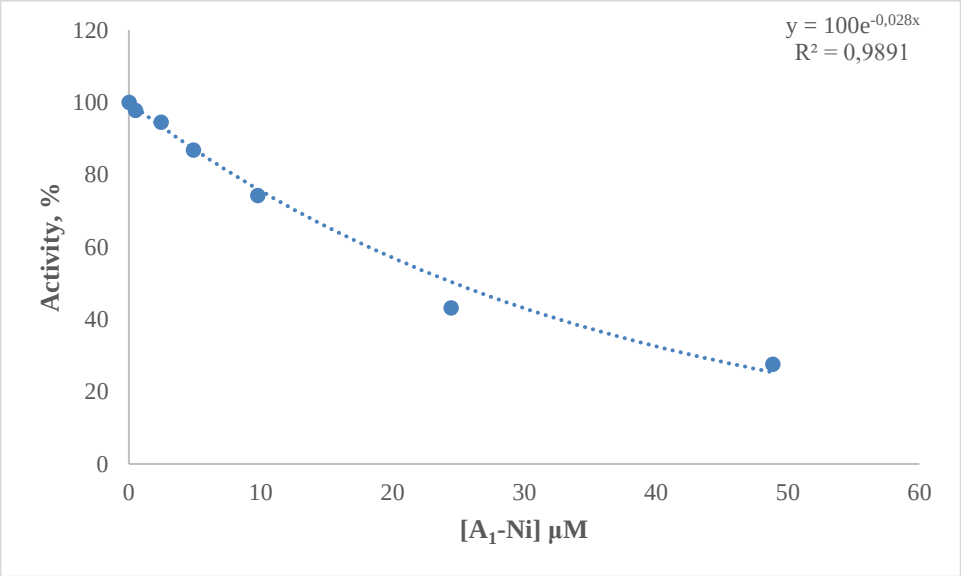


Figure 4.32. The effect of A_1-Ni complex on BChE activity.

The effect of (A_1-Ni) complex on BChE enzyme activity was examined in the range of 0.49-48.88 µM concentrations. According to the results obtained, the (A_1-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 24.76 µM (Figure 4.32).

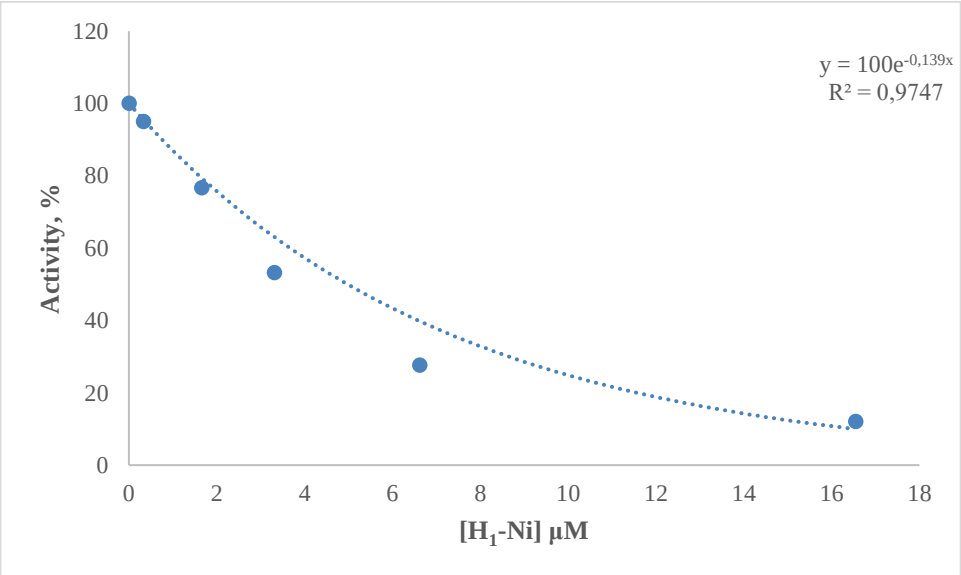


Figure 4.33. Impact of H_1-Ni complex on BChE activity.

The effect of (H_1-Ni) complex on BChE enzyme activity was examined in the range of 0.33-16.55 µM concentrations. According to the results obtained, the (H_1-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 4.99 µM (Figure 4.33).

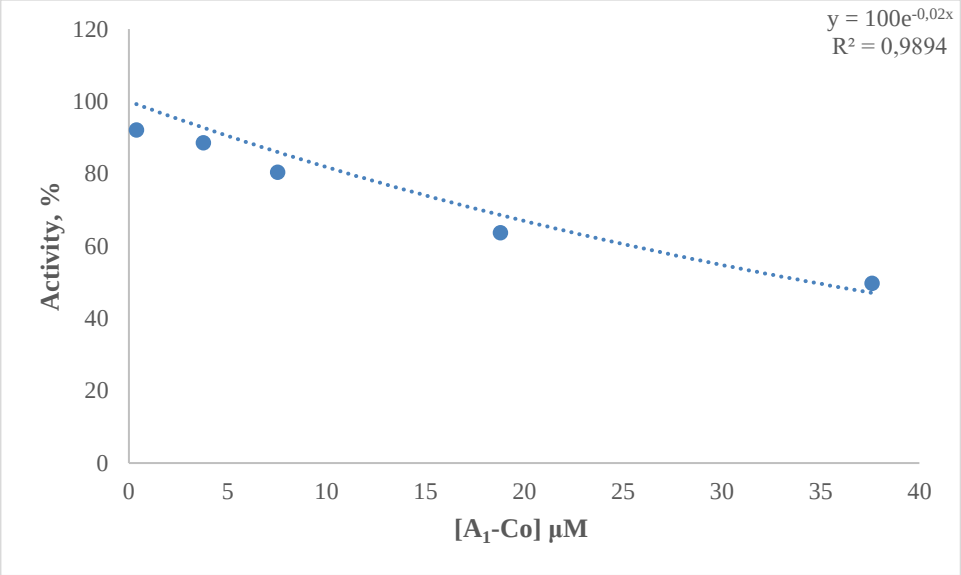


Figure 4.34. The effect of A_1-Co complex on BChE activity.

The effect of (A_1-Co) complex on BChE enzyme activity was examined in the range of 0.15-37.6 µM concentrations. According to the results obtained, the (A_1-Co) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 34.66 µM (Figure 4.34).

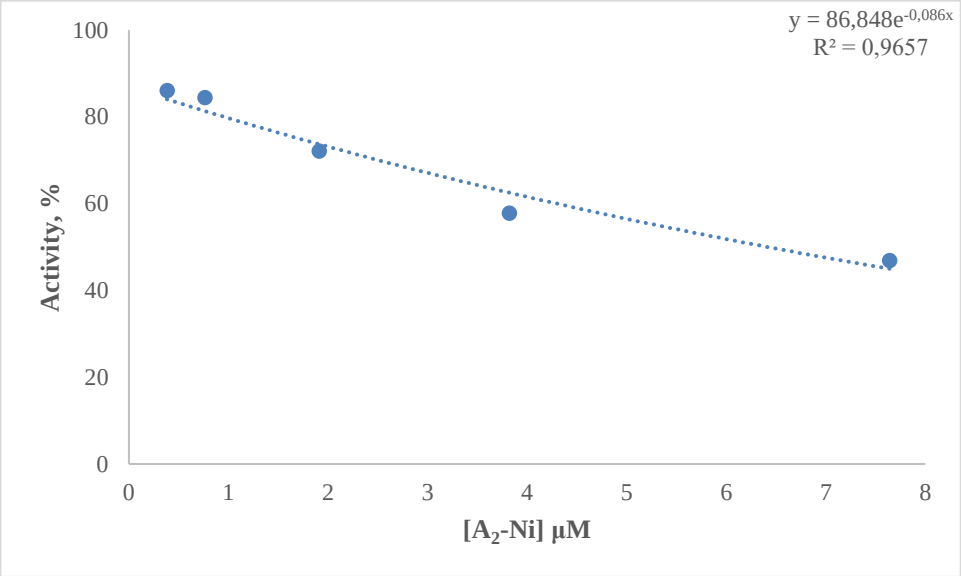


Figure 4.35. The effect of A_2-Ni complex on BChE activity.

The effect of (A_2-Ni) complex on Butyrylcholinesterase enzyme activity was examined in the range of 0.38-7.64 µM concentrations. According to the results obtained, the (A_2-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 6.42 µM (Figure 4.35).

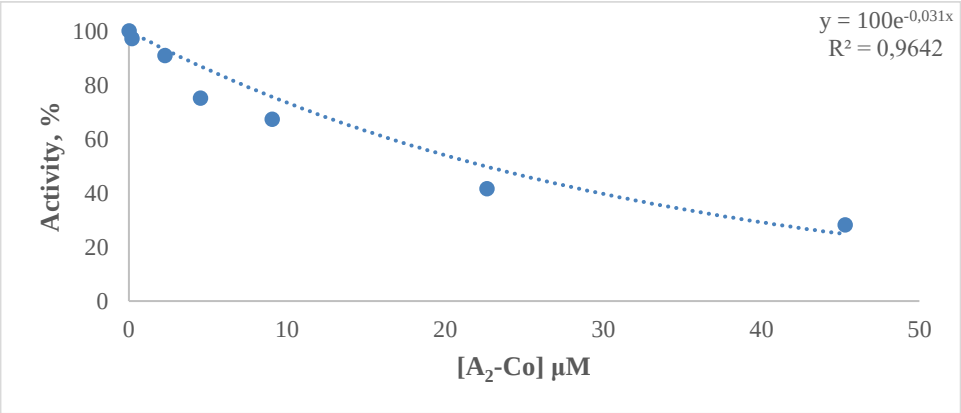


Figure 4.36. The effect of A_2-Co complex on BChE activity.

The effect of (A_2-Co) complex on BChE enzyme activity was examined in the range of 0.18-45.29 µM concentrations. According to the results obtained, the (A_2-Co) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 22.36 µM (Figure 4.36).

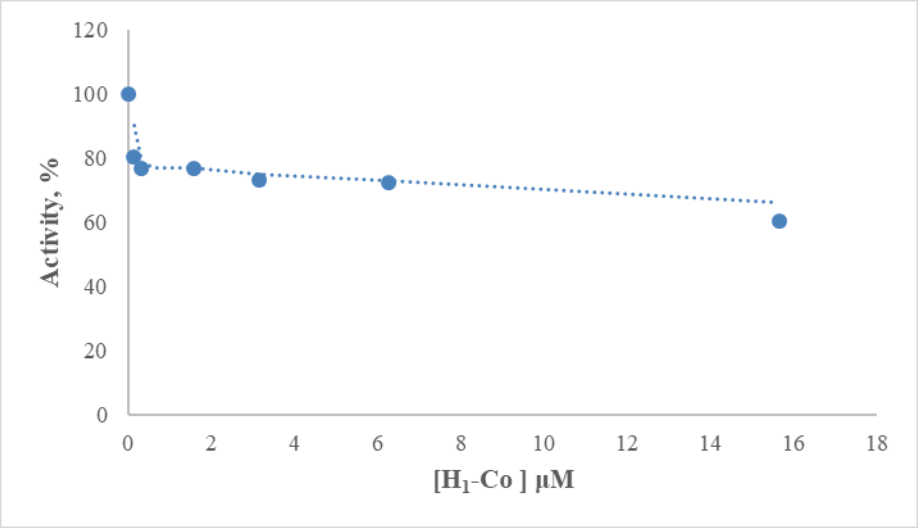


Figure 4.37. The effect of H_1- Co complex on pancreatic lipase activity.

The effect of (H_1- Co) complex was examined in the concentration range of 0.13-15.65 µM. According to the results obtained, a linear effect was not observed (Figure 4.37).

4.1.4. The Effect of Metal Complexes on pancreatic Lipase Enzyme Activity

Finally, the efficacy research with lipase yielded a positive result when the rapid inhibition that occurred at absorbance 408 nm was plotted against the percentage relative activity for each metal complex**.**

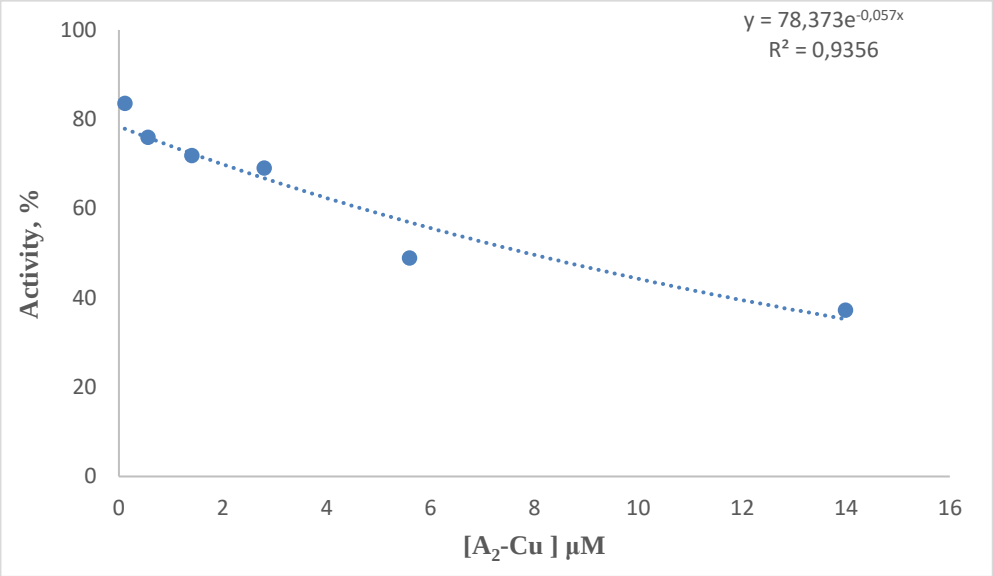


Figure 4.38. The effect of A_2-Cu complex on pancreatic lipase activity.

The effect of (A_2-Cu) complex on lipase enzyme activity was examined in the range of 0.56-14 µM concentrations. According to the results obtained, the (A_2-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 7.885 µM (Figure 4.38).

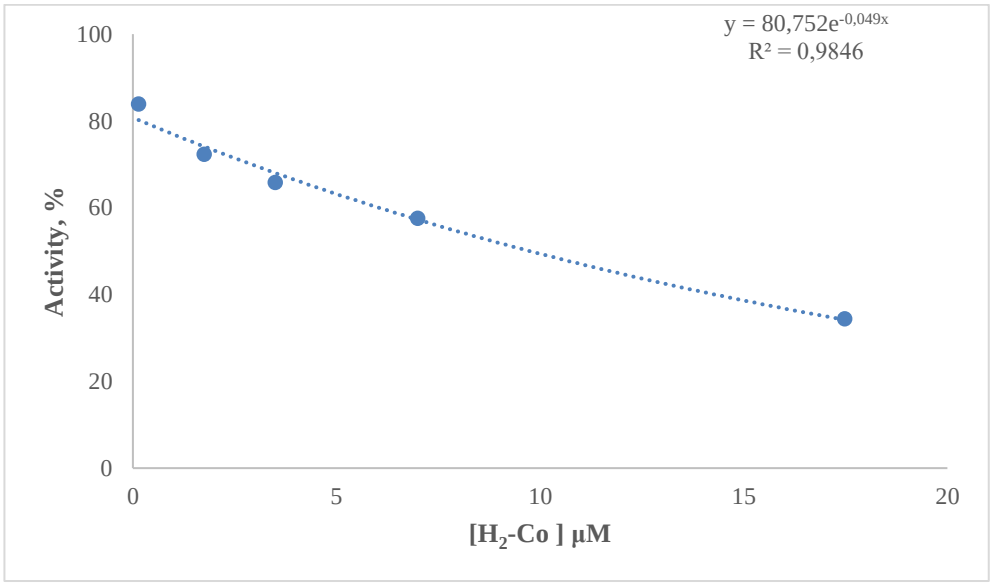


Figure 4.39. Impact of H_2-Co complex on pancreatic lipase activity.

The effect of (H_2-Co) complex on Lipase enzyme activity was examined in the range of 0.11-13.99 µM concentrations. According to the results obtained, the (H_2-Co)

complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 9.783 µM (Figure 4.39).

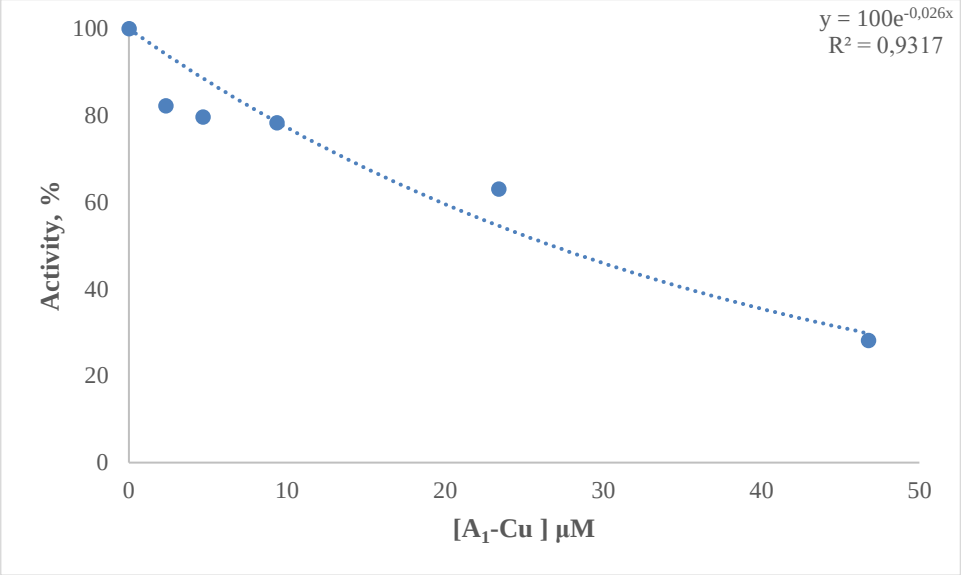


Figure 4.40. Effect of A_1-Cu complex on theaactivity of pancreatic lipase.

The effect of (A_1-Cu) complex on lipase enzyme activity was examined in the range of 2.34-46.77 µM concentrations. According to the results obtained, the (A_1-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 26.66 µM (Figure 4.40).

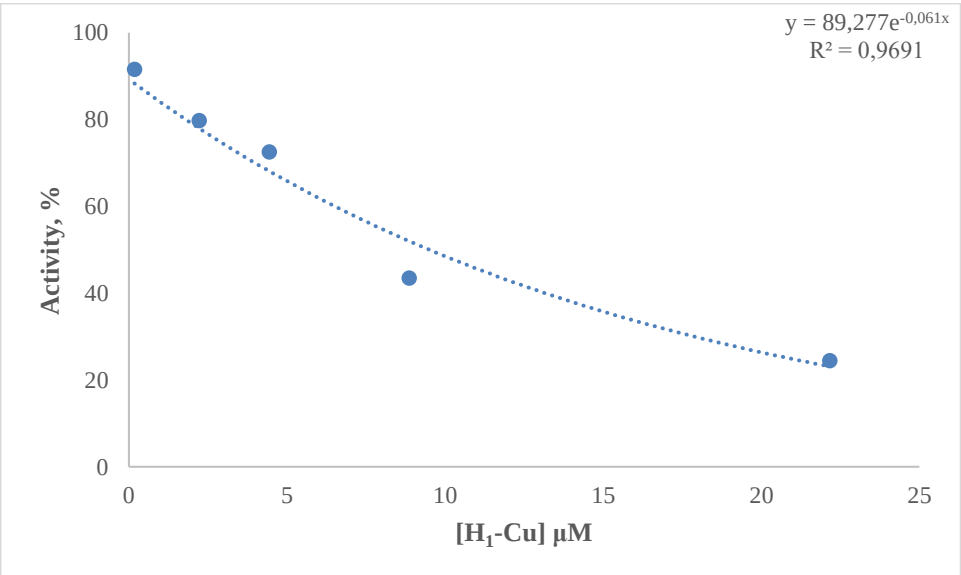


Figure 4.41. The effect of H_1- Cu complex on pancreatic lipase activity.

The effect of (H_1-Cu) complex on lipase enzyme activity was examined in the range of 0.18-22.16 µM concentrations. According to the results obtained, the (H_1-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 9.504 µM (Figure 4.41).

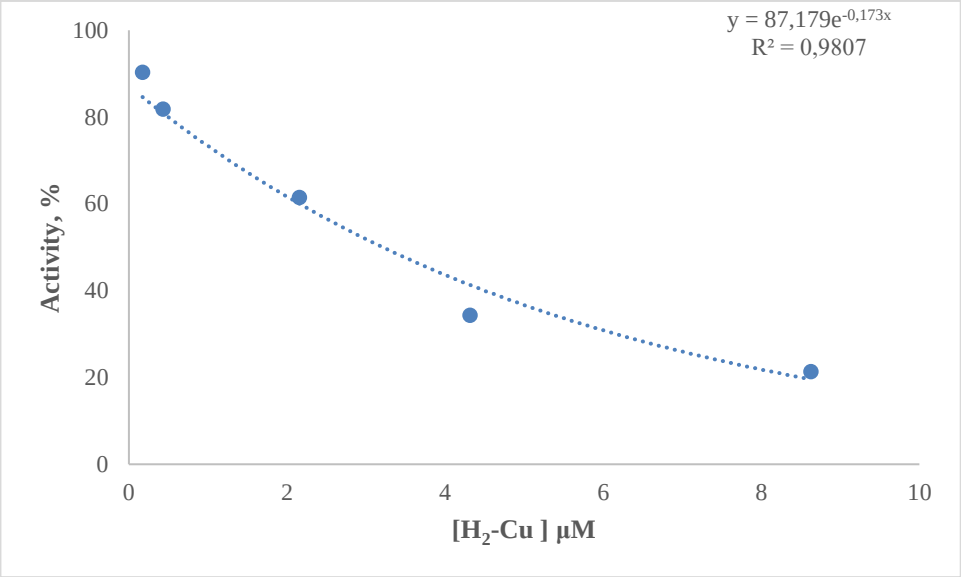


Figure 4.42. H_2-Cu complex modulation of pancreatic lipase activity.

The effect of (H_2-Cu) complex on lipase enzyme activity was examined in the range of 0.17-8.63 µM concentrations. According to the results obtained, the (H_2-Cu) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 3.214 µM (Figure 4.42).

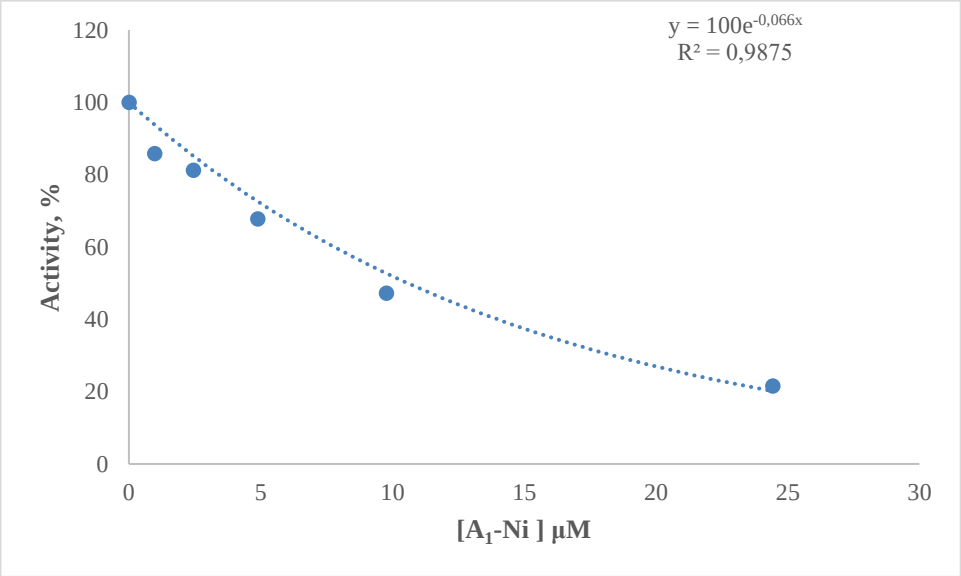


Figure 4.43. The effect of A_1- Ni complex on pancreatic lipase activity.

The effect of (A_1-Ni) complex on lipase enzyme activity was examined in the range of 0.97-24.44 µM concentrations. According to the results obtained, the (A_1-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 10.5 µM (Figure 4.43).

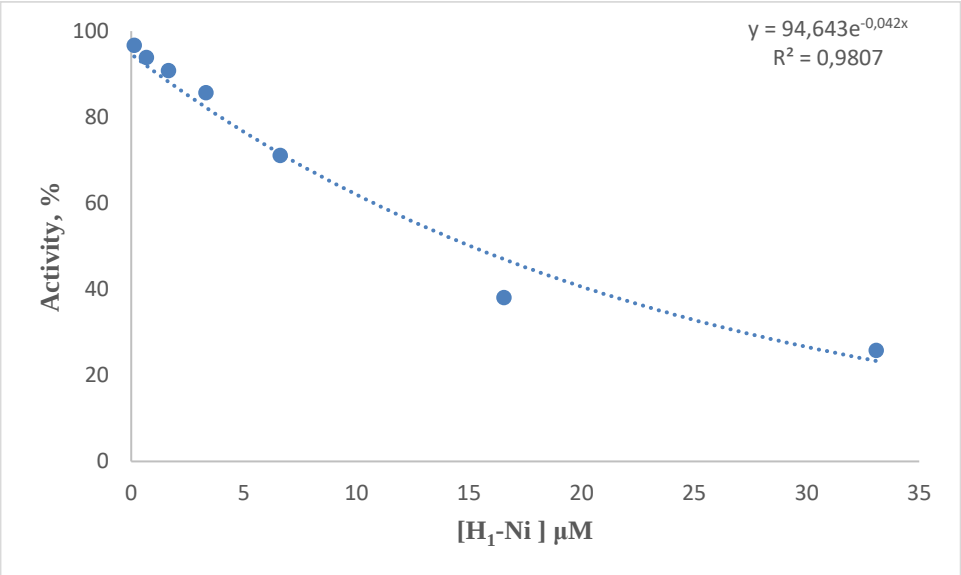


Figure 4.44. Impact of H_1-Ni complex on pancreatic lipase activity.

The effect of (H_1-Ni) complex on lipase enzyme activity was examined in the range of 1.32-33.09 µM concentrations. According to the results obtained, the (H_1-Ni) complex

inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 15.19 µM (Figure 4.44).

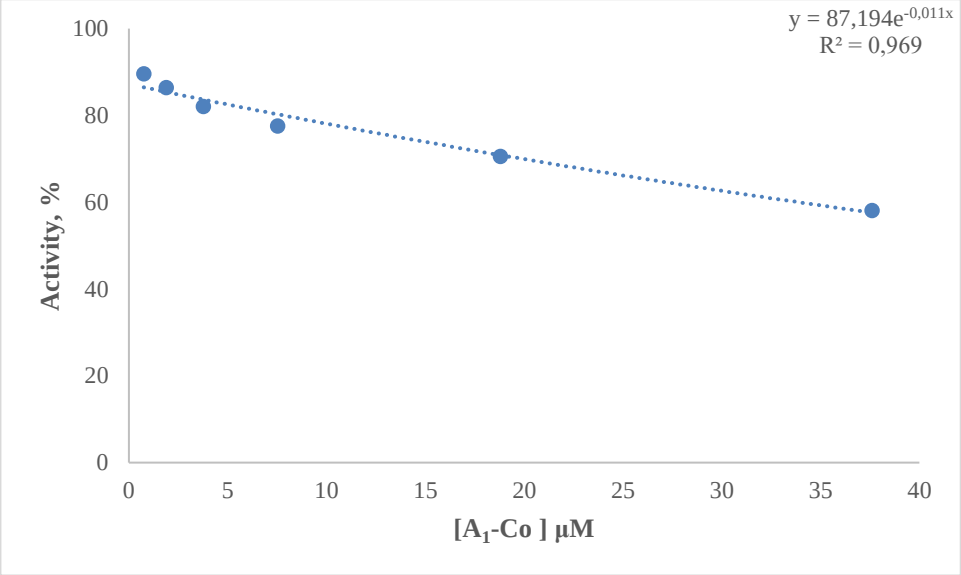


Figure 4.45. The effect of (A_1- Co) complex on pancreatic lipase activity.

The effect of (A_1-Co) complex on lipase enzyme activity was examined in the range of 0.75-37.6 µM concentrations. It was observed that in this concentration range, the complex could inhibit the enzyme by a maximum of 41.86% (Figure 4.45).

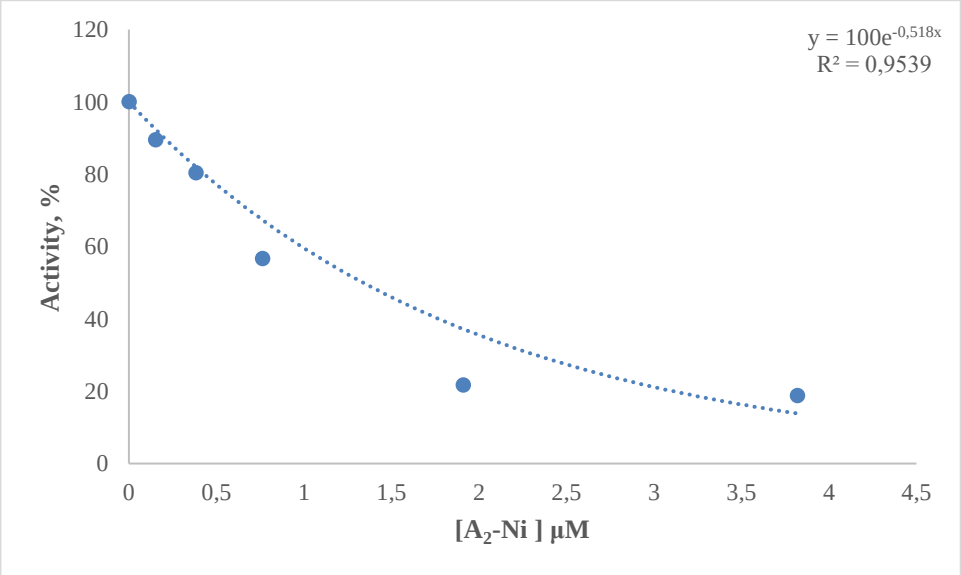


Figure 4.46. The effect of A_2- Ni complex on pancreatic lipase activity.

The effect of (A_2-Ni) complex on lipase enzyme activity was examined in the range of 0.15-3.82 µM concentrations. According to the results obtained, the (A_2-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 1.34 µM (Figure 4.46).

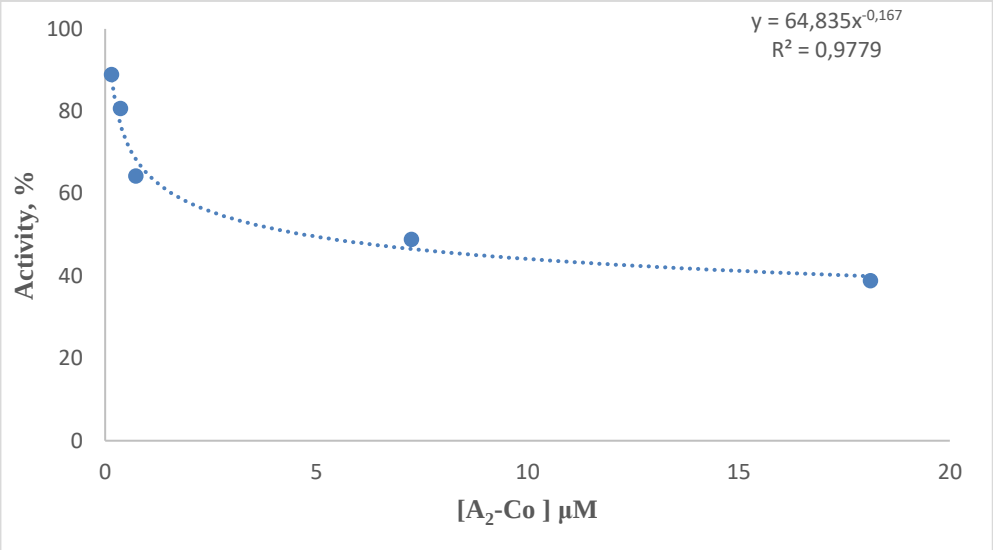


Figure 4.47. Influence of A_2-Co complex on pancreatic lipase enzyme function.

The effect of (A_2-Co) complex on lipase enzyme activity was examined in the range of 0.145-18.12 µM concentrations. According to the results obtained, the (A_2-Co) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 4.739 µM (Figure 4.47).

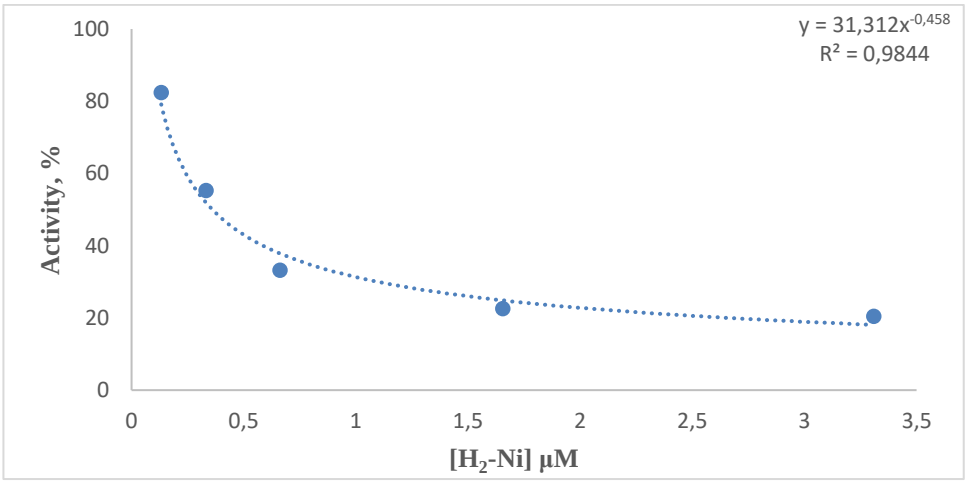


Figure 4.48. Impact of H_2-Ni complex on pancreatic lipase activity.

The effect of (H_2-Ni) complex on lipase enzyme activity was examined in the range of 0.132-3.31 μM concentrations. According to the results obtained, the (H_2-Ni) complex inhibited the enzyme. Using the graphic equation, the IC_{50} value for inhibition was calculated as 0.36 μM (Figure 4.48).

4.2. Docking Results

Within the scope of the study, a docking study was planned to reveal the interaction mechanisms of the metal complexes whose effects on enzyme activities were examined. However, due to the large molecular structures of the metal complexes, ligand preparation could not be done using the docking program. For this reason, docking studies were carried out only for the ligands from which the complexes showing the most inhibitory effects were synthesized.

The complex that showed the strongest inhibitory effect on the AChE enzyme was H_2-Ni with an IC_{50} value of 1.03 μM. For this reason, docking study of AChE and H_2 ligand was performed, and the resulting poses are given below (Figure 4.49-52).

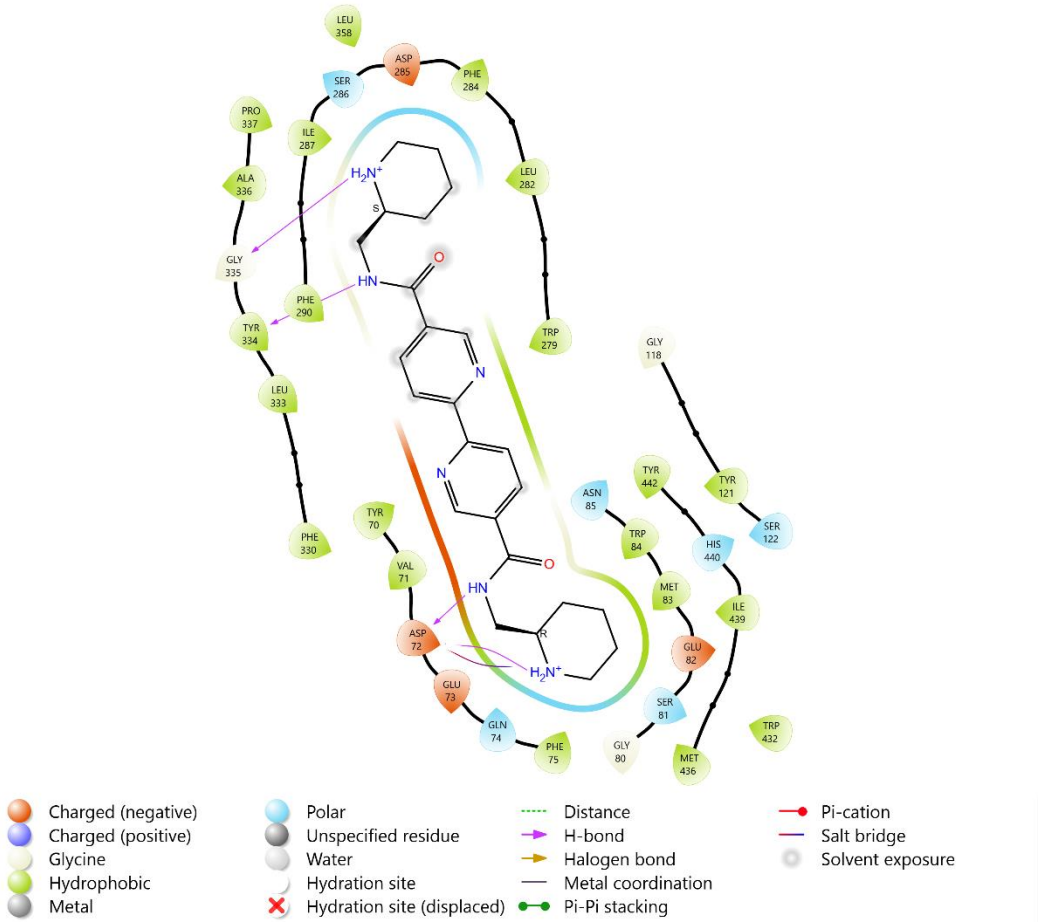


Figure 4.49. 2D image of the Interaction of AChE with the H_2 Ligand.

Hydrophobic interaction occurred between the ligand and residues TYR70, VAL71, PHE75, MET83, TRP84, TYR121, TRP279, LEU282, PHE284, ILE287, PHE290, PHE330, LEU333, TYR334, ALA336, PRO337, LEU358, TRP432, MET436, ILE439, and TYR442. Charged (negative) interaction occurred with residues ASP72, GLU73, GLU82 and ASP285, polar interaction occurred with residues GLN74, SER81, ASN85, SER122, SER286 and HIS440. H-bond occurred with ASP72, TYR334 and GLY335, and salt bridge occurred with ASP72. It was observed that the docking score of the ligand-enzyme interaction was -11.053, and this value was better compared to the value of tacrine (-9.670), the standard inhibitor of the enzyme.

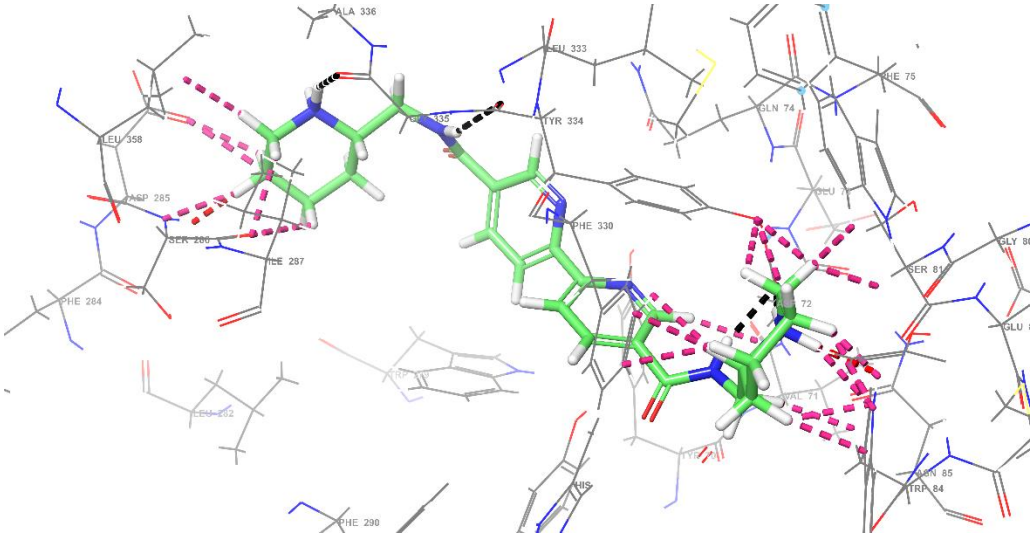


Figure 4.50. 3D Image of the interaction of AChE with the H_2 ligand.

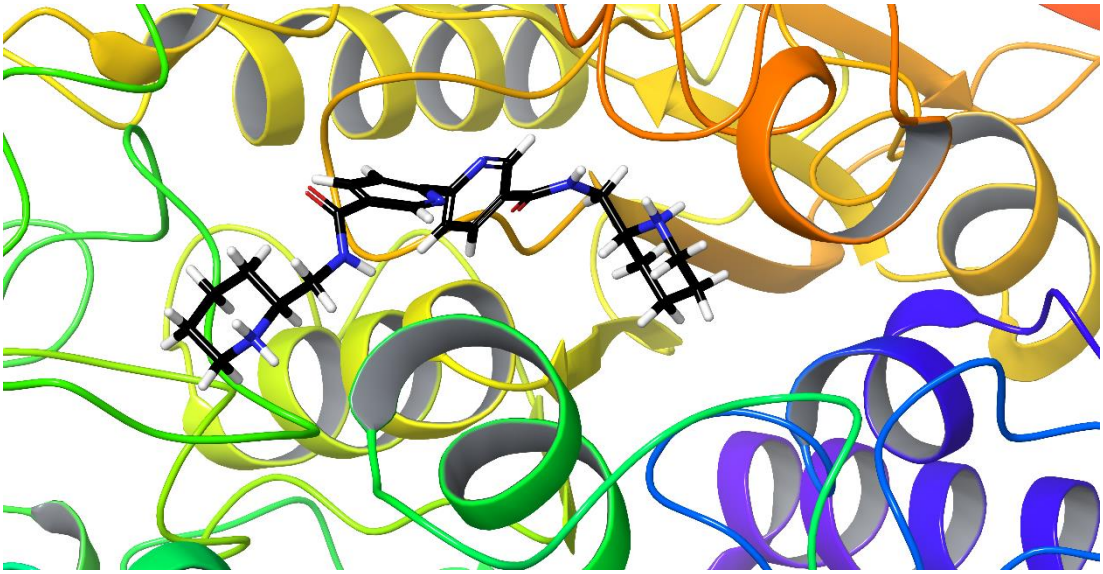


Figure 4.51. Ribbon structure image of the interaction of AChE enzyme with the H_2 ligand.

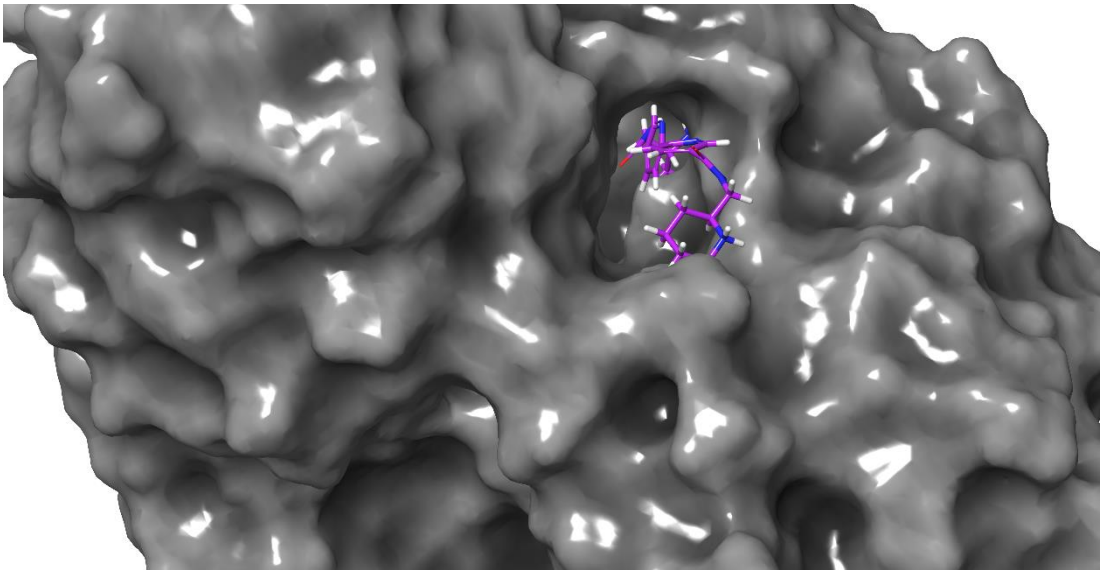


Figure 4.52. Surface image of the interaction of AChE with the H_2 ligand.

The complex that showed the strongest inhibitory effect on the BChE enzyme was H_2-Ni with an IC_{50} value of 4.53 µM. For this reason, docking study of BChE and H_2 ligand was performed, and the resulting poses are given below (Figure 4.53-56).

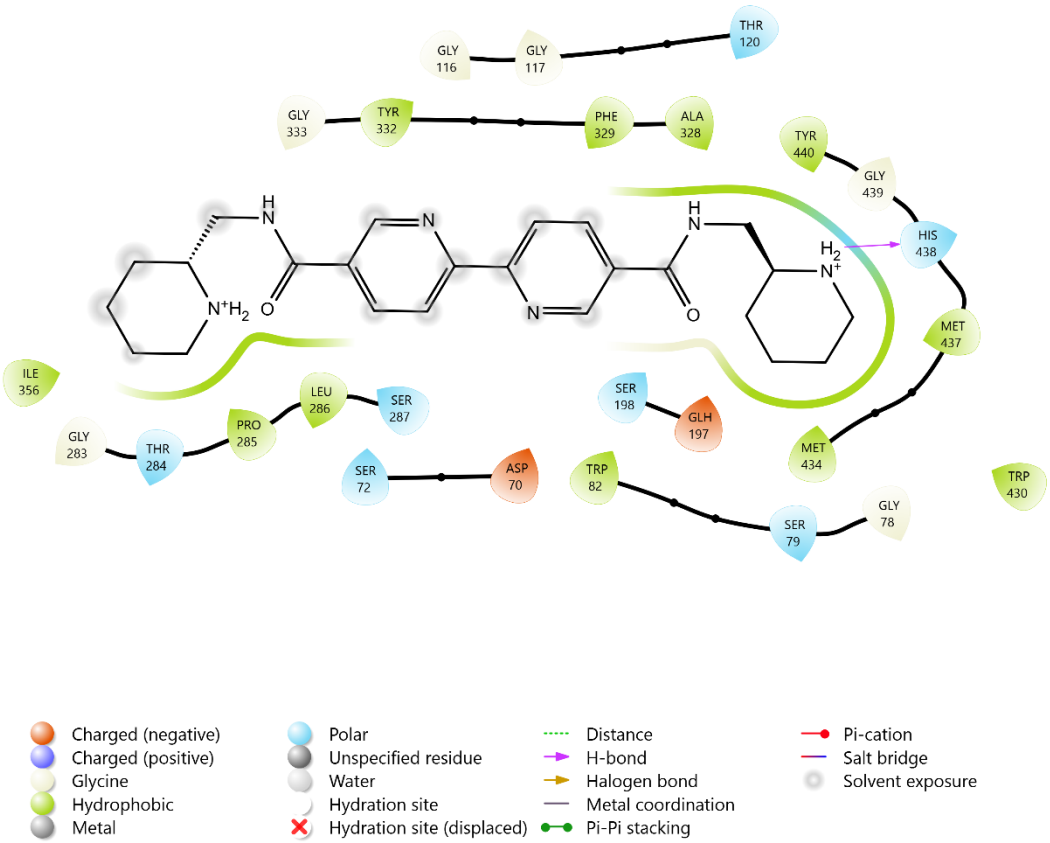


Figure 4.53. 2D image of the interaction of BChE with the H_2 ligand.

Hydrophobic interaction occurred between the ligand and residues TRP82, PRO285, LEU286, TYR332, PHE329, ALA328, ILE356, TRP430, MET434, MET437, and TYR440. Charged (negative) interaction occurred with residues ASP70 and GLH197, polar interaction occurred with residues SER72, SER79, THR120, SER198, THR284, SER287, and HIS438. H-bond occurred with HIS438. It was observed that the docking score of the ligand-enzyme interaction was -4.825, and this value was lower compared to the value of tacrine (-6.913), the standard inhibitor of the enzyme.

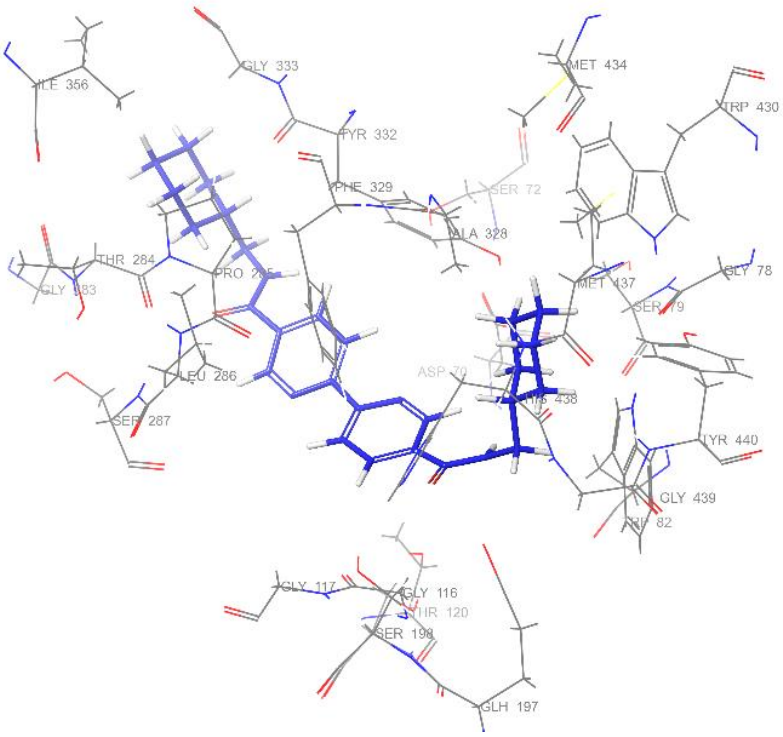


Figure 4.54. 3D image of the interaction of BChE with the H_2 ligand.

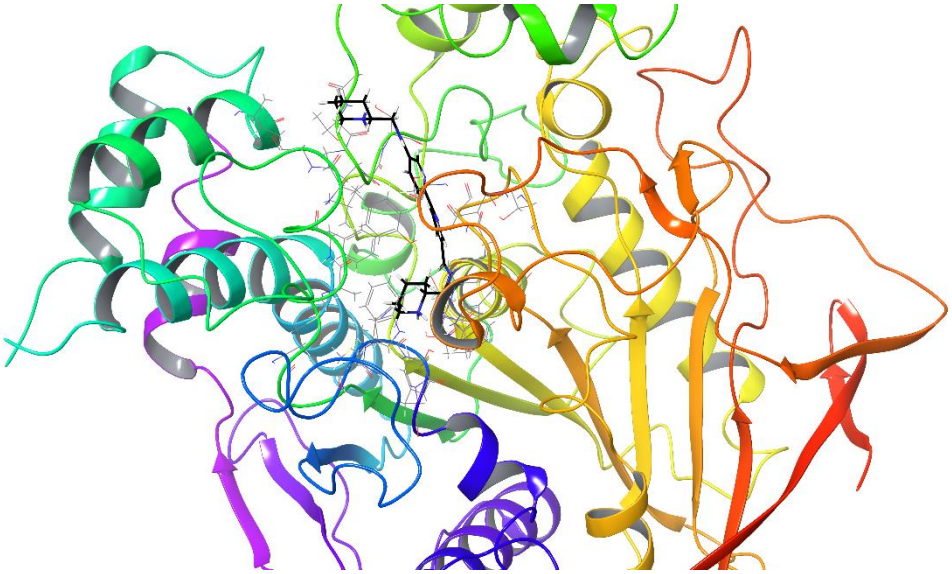


Figure 4.55. Ribbon structure image of the interaction of BChE enzyme with the H_2 ligand.

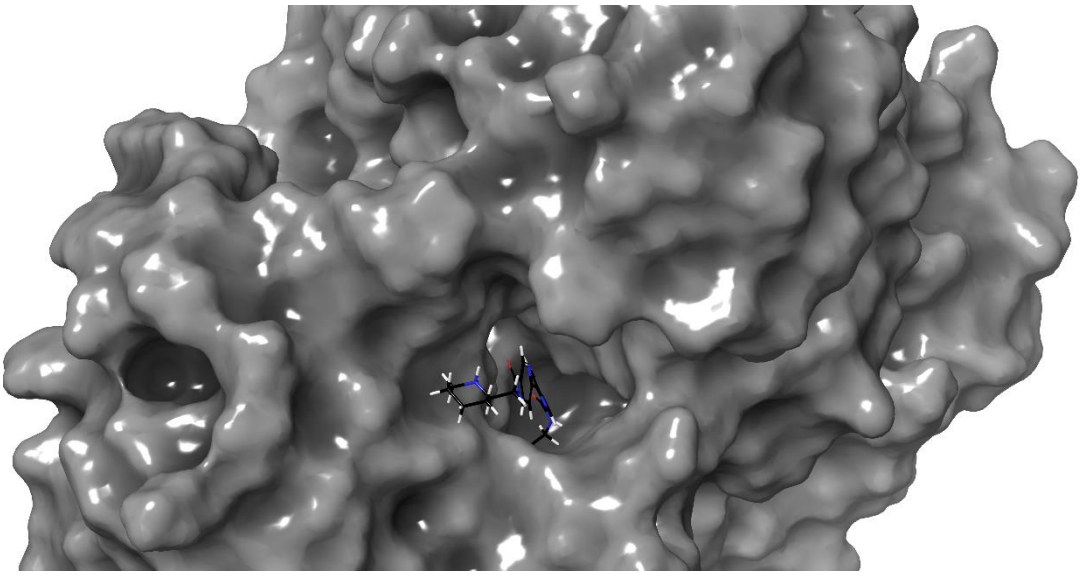


Figure 4.56. Surface image of the interaction of BChE with the H_2 ligand.

The complex that showed the strongest inhibitory effect on the lipase enzyme was H_2-Ni with an IC_{50} value of 0.36 μM. For this reason, docking study of lipase and H_2 ligand was performed, and the resulting poses are given below (Figure 4.57-60).

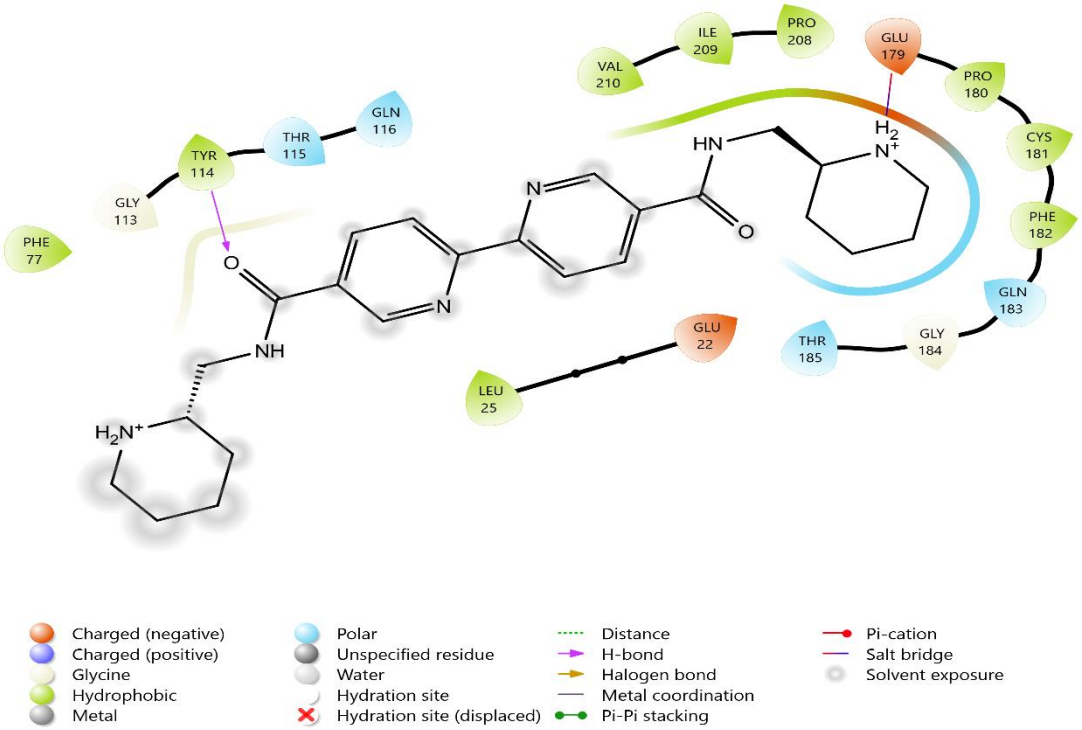


Figure 4.57. 2D image of the interaction of lipase with the H_2 ligand.

Hydrophobic interaction occurred between the ligand and residues LEU25, PHE77, TYR114, PRO180, CYS181, PHE182, PRO208, ILE209, and VAL210. Charged (negative) interaction occurred with residues GLU22 and GLU179, polar interaction occurred with residues THR115, GLN116, GLN183, and THR185. The salt bridge occurred with GLU179. It was observed that the docking score of the ligand-enzyme interaction was -2.774, and this value was higher compared to the value of methoxy phosphinic acid (-0.863), the standard inhibitor of the enzyme.

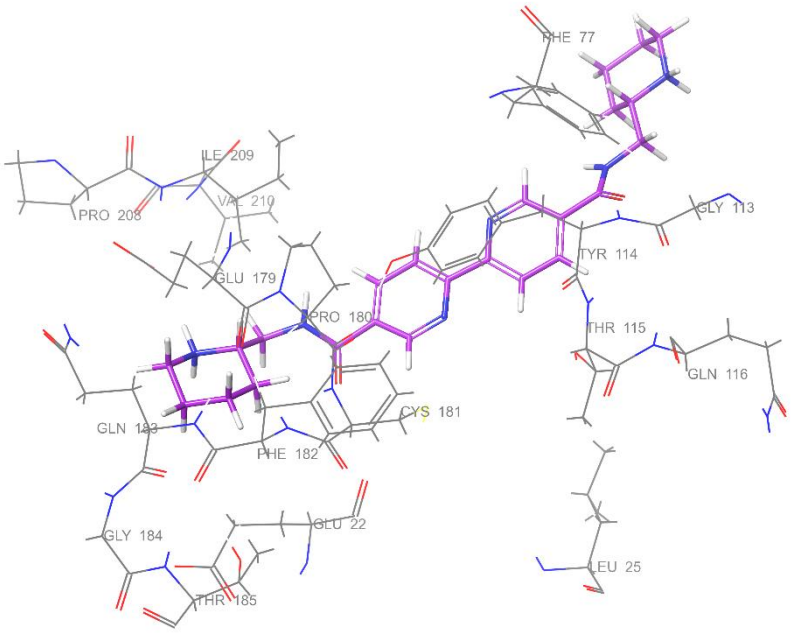


Figure 4.58. 3D image of the interaction of lipase with the H_2 ligand.

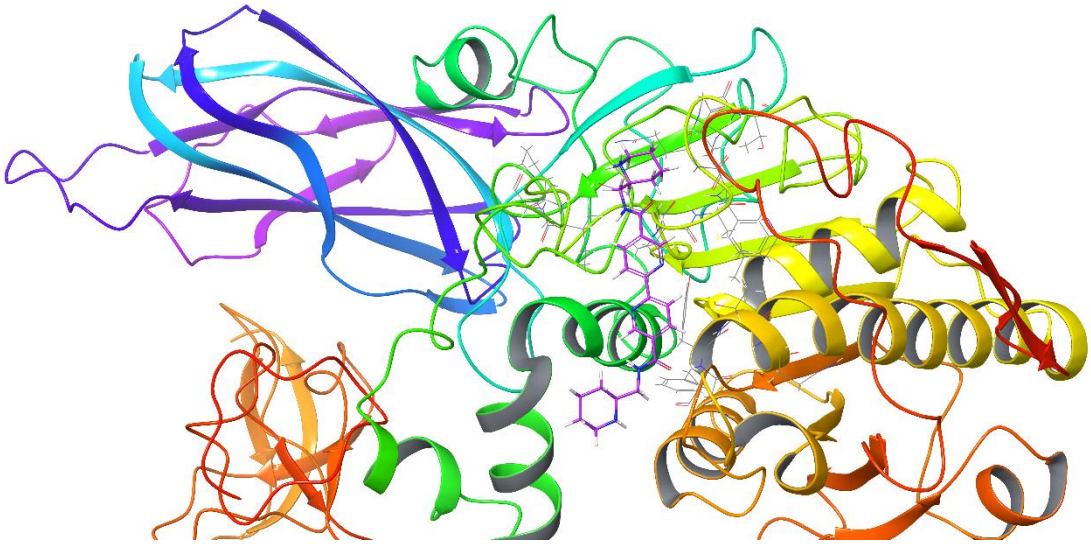


Figure 4.59. Ribbon structure image of the interaction of lipase with the H_2 ligand.

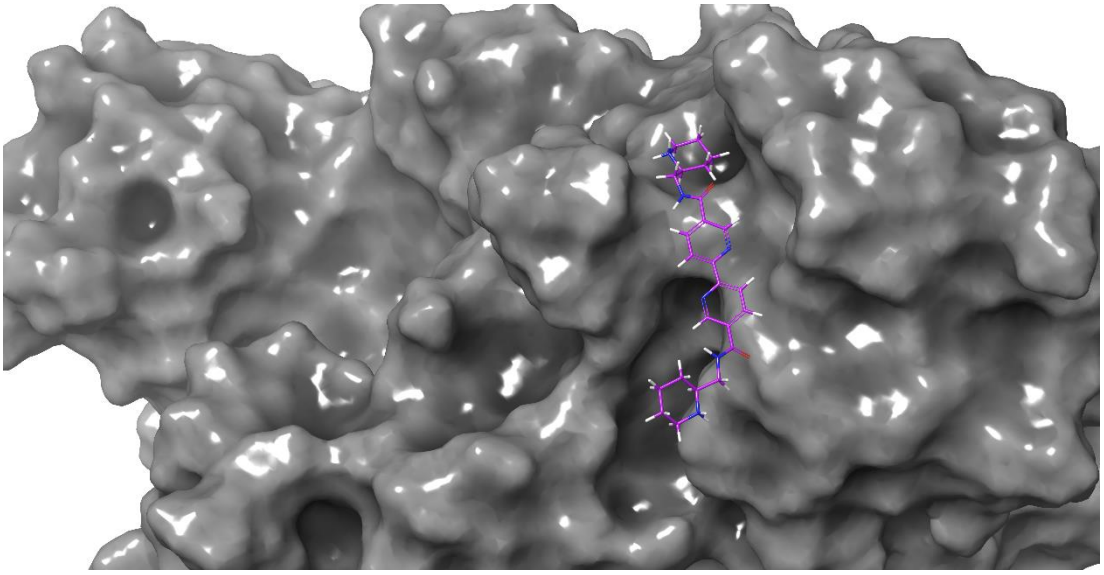


Figure 4.60. Surface image of the interaction of lipase with the H_2 ligand.

Table 4.1. Docking scores (kcal) obtained for ligands and standard inhibitors of enzymes.

		docking score	XP GScore	glide emodel
AChE	H2	-11.053	-11.053	-82.296
	Tacrine	-9.670	-9.671	-46.072
BChE	H2	-4.825	-4.825	-63.002
	Tacrine	-6.913	-6.913	-42.707
Lipase	H2	-2.774	-2.775	-39.718
	methoxy phosphinic acid	-0.863	-863	-20.917

SECTION 5

DISCUSSION

This thesis aims to investigate the impact of copper, nickel, and cobalt metal complexes on the activity of several key enzymes: pancreatic lipase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase. These enzymes play significant roles in metabolic processes and neurological functions, making them critical targets for therapeutic interventions. The study findings reveal that the metal complexes of copper, nickel, and cobalt exhibit significant inhibitory effects on acetylcholinesterase, pancreatic lipase, and butyrylcholinesterase, as demonstrated by their IC50 values, which indicate the concentration of inhibitor required to reduce enzyme activity by 50%. This suggests that these metal complexes have the potential to modulate enzyme activity effectively. Metal complexes and enzyme activity are essential components in the human body, contributing to a range of biological processes. The study highlights the potential of these metal complexes to exhibit various beneficial properties, including anti-obesity, anti-diabetic, anticancer, and anti-cardiovascular effects. These properties make them promising candidates for developing new therapeutic agents. In the context of chronic diseases, enzyme inhibition is a crucial area of research. Chronic conditions such as obesity, diabetes, cancer, atherosclerosis, and cardiovascular diseases often involve dysregulated enzyme activity. Investigating the enzyme-substrate interactions in these conditions is vital for understanding the disease mechanisms and developing effective treatments. The presence of organic chemicals, inorganic metals, or biosynthetic molecules can significantly influence these interactions through covalent or noncovalent binding to the enzyme's active site. This thesis emphasizes the importance of understanding enzyme inhibition in the development of therapeutic strategies for various chronic diseases. By exploring the inhibitory effects of copper, nickel, and cobalt metal complexes on specific enzymes, this study contributes to the broader knowledge of how metal complexes can be

utilized in medical applications to regulate enzyme activity and improve health outcomes [39].

α-Glucosidase inhibition offers therapeutic benefits by slowing carbohydrate digestion, thereby reducing glucose absorption and aiding in blood sugar regulation. This makes it a crucial target for managing non-insulin-dependent diabetes mellitus. Bavachalcone, chemically known as 1-[2-hydroxy-4-methoxy-5-(3-methylbut-2-enyl) phenyl]-3-(4-hydroxyphenyl) prop-2-en-1-one, exhibits various pharmacological activities, including inhibiting bone cell differentiation and possessing bacteriostatic, anti-inflammatory, antiviral, and antitumor properties. Chalcones, the chemical class to which bavachalcone belongs, are open-chain flavonoids characterized by two aromatic rings connected by a three-carbon α,β-unsaturated carbonyl system. These vibrant orange compounds are prevalent in many plant parts, especially flowers, and are extracted from the traditional Chinese medicinal plant Fructus psoraleae. In studies assessing bavachalcone's effectiveness in inhibiting α-glucosidase, acarbose served as the positive control. Results showed that as the concentration of bavachalcone increased, its inhibitory effect on α-glucosidase also rose. Specifically, at 30 μg/ml, bavachalcone achieved a 75.36% inhibition rate. Plant extracts rich in chalcones and flavonoids are noted for their chemical stability and ability to inhibit α-glucosidase. Phenolidin and its polymers have shown significant inhibitory effects on α-glucosidase, with IC50 values of 0.21 mg/ml and 0.12 mg/ml, respectively. Docking studies using Auto Dock revealed a binding free energy of -6.26 kcal/mol for the bavachalcone-α-glucosidase complex, suggesting a strong binding affinity. These findings are similar to those observed with phenolic acids, which interact hydrophobically with aromatic residues in α-glucosidase such as Trp391, Trp710, Trp715, Trp789, Phe385, Phe389, Phe444, and Phe786, and form robust hydrogen bonds with Trp391 and Asp392. These interactions are critical for the inhibition of α-glucosidase activity. [41]. All play an important role in the resulting inhibition of α-glucosidase activity.

In an earlier investigation, α-glucosidase was extracted from Penicillium chrysogenum, with its activity boosted by plant growth regulators and inhibited by dansyl chloride. The enzyme's activity was found to be stimulated by Ca^{2+} and Mg^{2+} ions, but it was inhibited by heavy metals such as Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, and Hg^{2+}. For immobilization, the enzyme was fixed onto Ca-alginate and then tested against aqueous extracts of Datura stramonium, Trigonella foenum-graecum, Hyoscyamus muticus, and Cynodon dactylon. The inhibition effectiveness of these extracts was quantified, with IC50 values determined to be 59.1, 73.6, 68.5, and 77.1 μg/ml, respectively [42].

Sohrabi et al. explored the inhibitory effects of cobalt (II) complexes containing 2-acetylbenzofuran hydrazide moieties on α-glucosidase. These complexes demonstrated greater activity compared to their free ligand counterparts and were classified as moderate inhibitors of the enzyme, with IC50 values ranging from 66.48 to 153.23 μM. Notably, the most potent inhibitor among these was a manganese (II) complex, which showed an IC50 of 66.48 μM, significantly lower than the 378.25 μM IC50 of acarbose, a standard reference compound. The study also evaluated several manganese (II) complexes with 2-acetylbenzofuran hydrazones, specifically [Mn(L1)2]Cl2, [Mn(L2)2]Cl2, [Mn(L3)2]Cl2, [Mn(L4)2]Cl2, [Mn(L5)2]Cl2, and [Mn(L7)2]Cl2. Among these, the complex with ligand L1 exhibited the highest inhibitory activity against α-glucosidase, with an IC50 of 45.63 μM. In contrast, the free ligand L1 did not inhibit the enzyme, highlighting the importance of the metal center in the complex for effective inhibition. This suggests that the electronic or structural properties conferred by the metal centre play a crucial role in enzyme inhibition. Complexes containing ligands L4 and L5 showed minimal activity, indicating that these specific configurations did not facilitate effective interactions with the enzyme. [43].

In light of prior studies, the rising incidence of diabetes has driven the pursuit of new pharmaceutical treatments. Over the past few decades, triazole and its derivatives have gained considerable attention for their pharmacological potential. Recent research aimed to evaluate whether triazoles and thiadiazoles, incorporating the lipophilic 4-

methylthiobutyl group synthesized from essential oil, contribute to inhibitory activity against diabetic enzymes. The primary compound identified in the fruit, stem, and root of the studied plants was erucin, comprising 96.6%, 85.3%, and 83.7% of the essential oil content, respectively. Essential oils extracted from these plant parts exhibited strong inhibitory effects on both α-glucosidase and α-amylase. For root extracts, the IC50 values were 0.81 ± 0.02 μg/mL against α-glucosidase and 0.13 ± 0.01 μg/mL against α-amylase. Significantly, derivatives 1b, 2b, 3b, and 2c demonstrated notable inhibitory activity against α-glucosidase, with IC50 values ranging from 0.49 to 1.43 μM, surpassing the performance of acarbose. This suggests that these derivatives, enhanced by their specific structural features, hold promise as effective inhibitors for managing diabetes. [44].

Alqahtani et al. investigated the antidiabetic potential of 3-oxolupenal and katononic acid from Nuxia oppositifolia through enzyme inhibition assays targeting α-amylase and α-glucosidase. The study found that 3-oxolupenal had an IC50 value of 46.2 μg/mL (101.6 μM) against α-amylase, while katononic acid had an IC50 of 52.4 μg/mL (119.3 μM). For α-glucosidase inhibition, 3-oxolupenal demonstrated greater potency with an IC50 of 62.3 μg/mL (141.9 μM), compared to katononic acid's IC50 of 88.6 μg/mL (194.8 μM). Molecular docking studies and fluorescence quenching experiments were conducted to further investigate the interactions between these compounds and the enzymes. The results showed that both 3-oxolupenal and katononic acid formed strong interactions with the active site residues of α-amylase and α-glucosidase. Fluorescence quenching data corroborated the high affinity of these compounds for both enzymes.

Overall, this research suggests that Nuxia oppositifolia holds promise for the treatment of type 2 diabetes mellitus, particularly due to the inhibitory effects of 3-oxolupenal and katononic acid on critical enzymes involved in glucose metabolism [45].

In this study, we investigated the impact of metal complexes (Cu, Ni, Co) on the α-glucosidase enzyme, which plays a pivotal role in starch breakdown and absorption in the intestine. Inhibiting this enzyme could lead to a significant reduction in the elevation of blood glucose levels, presenting a potentially crucial strategy for

managing hyperglycemia in type 2 diabetes. We aimed to explore the in vitro anti hyperglycemic effects of these metal ligands, chosen as the focus of our research.

However, upon conducting evaluations of these compounds for enzyme inhibition and activation, we observed no discernible effect on α-glucosidase enzymes. When compared with findings from previous studies, our results suggest that the effects of metal complexes vary depending on concentration. Importantly, we found that within the investigated concentration range, the complex did not exhibit a linear effect on the enzyme.

Acetylcholine serves as a vital biomarker in Alzheimer's disease, governing functions in both the central and autonomic nervous systems. It plays a crucial role in facilitating communication between nerve cells, particularly in memory and learning processes. The cholinergic hypothesis underscores the significance of the acetylcholinesterase enzyme in regulating acetylcholine levels, essential for various cerebral functions including development, blood flow, sleep, memory, learning, and cognition. Acetylcholine transmits warning signals through muscarinic or nicotinic receptors. In Alzheimer's disease, blocking acetylcholinesterase and butyrylcholinesterase enzymes at cholinergic synapses and neuromuscular junctions can potentially enhance acetylcholine density, which typically decreases with increased enzyme activity. In drug development, the AChE enzyme is prioritized due to its structure and direct association with acetylcholine, whereas the BChE enzyme gains significance for inhibition, especially in advanced conditions where its levels are elevated. A previous study highlighted N-trans feruloyldopamine as the most potent inhibitor of AChE, with the highest IC50 value (8.52 μM), while caffeic acid exhibited the lowest inhibitory effect with an IC50 of 21.81 μM. [47].

Stellenboom conducted a study focusing on the inhibition effects of E/Z-bisPMB and Z-bisPMB on human carbonic anhydrase isozymes I and II (hCA I and II), as well as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Both E/Z-bisPMB and Z-bisPMB displayed similar inhibition profiles against all four enzymes tested, with IC50 values ranging from 10.9 to 439.7 nM and Ki values ranging from 5.4 to

195.4 nM. Notably, bisPMB exhibited greater potency in inhibiting CA I, CA II, and AChE compared to commercially available inhibitors [48].

In a prior study, a new ligand identified as 3-hydroxybenzaldehyde-4-nitrobenzoic acid hydrazone, along with its corresponding Nickel (II) and Copper (II) complexes, was synthesized and characterized using various spectroscopic techniques. The investigation into the acetylcholinesterase enzyme delineated three distinct domains: esteratic, anionic, and hydrophobic. Previous research on cholinesterase inhibitors underscored the significance of hydrophobicity, electronic effects, and steric contributions, as supported by phenserine studies. The ligand and its metal (II) complexes exhibited moderate inhibition of cholinesterase activity. Enhanced hydrophobicity correlated with increased efficacy, particularly notable in the hydrazone ligand, which displayed structural similarities to physostigmine and boasted an IC50 value of 190 ± 20 μg/mL. Notably, the flexible N-N bond in the ligand facilitated effective interactions with the enzyme-inhibitor complex. In contrast, the Nickel (II) and Copper (II) complexes, lacking a free carbonyl group, demonstrated lower anticholinesterase activity, with IC50 values of 390 ± 80 μg/mL and 220 ± 20 μg/mL, respectively. These findings underscore the importance of structural elements in modulating inhibitory activity against acetylcholinesterase, providing valuable insights for potential therapeutic applications. [49].

Budryn and colleagues conducted an analysis of 16 hydroxybenzoic acids as potential inhibitors of acetylcholinesterase (AChE). These compounds were observed to impede the hydrolysis of acetylcholine, with methyl syringinate showing the most promising characteristics by acting as a competitive inhibitor at the catalytic site. Additionally, the studied chemicals displayed inhibition of β-amyloid plaque formation, potentially through interactions with anionic or peripheral binding sites. The hydroxybenzoic acids exhibited a range of IC50 values (from 5.50 to 34.19 μmol/μmol of AChE) and binding constants (Ka ranged from 20.53 to 253.16 L/mol), indicating reversible actions and activity at physiological doses without inducing toxicity.
This suggests their potential utility as non-toxic, reversible inhibitors with therapeutic relevance. [50].

Based on our research findings, we undertook an investigation into the impact of metal complexes, specifically cobalt (Co), nickel (Ni), and copper (Cu) complexes, on acetylcholinesterase activity. Our study revealed distinct inhibitory effects of each metal complex on the acetylcholinesterase enzyme. The observed variations in inhibition rates among the Co, Ni, and Cu metal complexes suggest that the specific metal and its coordination with ligands significantly influence their inhibitory activity on acetylcholinesterase. Understanding these differences in inhibition rates provides valuable insights into the potential applications and mechanisms of these metal complexes in enzyme inhibition and neurobiology. Among the tested metal complexes, H2-Ni demonstrated the most potent inhibition of acetylcholinesterase, with an IC50 of 1.03 μM, indicating its strong ability to inhibit the enzyme. Conversely, A2-Co exhibited the lowest inhibition, with an IC50 of 24.76 μM. These results highlight the varying efficacy of different metal complexes in inhibiting acetylcholinesterase activity. Further exploration, considering factors such as concentration, reaction kinetics, and comparisons with existing literature, will contribute to a comprehensive understanding of the implications of these metal complexes in biological systems.

Butyrylcholinesterase (BChE), are responsible for the hydrolysis of ACh within the brain is primarily attributed to these cells. ChE inhibitors can elevate ACh levels in the brain, as they hydrolyze ACh but can also hydrolyze other molecules. In animal studies, selective AChE inhibitors increased ACh levels 16-fold, while specific BuChE inhibition resulted in a 5-fold increase. This shows that AChE is responsible for the majority of ACh hydrolysis in the healthy brain, whereas BuChE takes over in the AChE-deficient brain. In Alzheimer's disease, AChE levels in the brain gradually decline, but BChE activity remains constant or increases up to 165% of normal levels. Potentially result, BChE has been offered as a therapeutic target for treating Alzheimer's disease in its advanced phases [51].

In a previous study, the focus was on evaluating the *in vitro* effects of ZINC390718 on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Molecular dynamics (MD) simulations were employed to characterize the binding mode of the compound within these enzymes. The investigation extended to exploring the *in vitro*

cytotoxicity of ZINC390718 using a primary astrocyte-enriched glial cell culture. ZINC390718 exhibited dual inhibitory activity against both AChE and BChE, with AChE inhibition occurring at a high micromolar range, IC_{50}=543.8 μM, and BChE inhibition at 241.1 μM. The inhibitory activity displayed concentration-dependency, with greater potency against BChE. In MD simulations, ZINC390718 interacted with catalytic residue sites on both enzymes through hydrophobic and hydrogen bonding interactions. Key residues involved in these interactions were identified for AChE (Leu67, Trp86, Fhe123, Tyr124, Ser293, Fhe295, and Tyr341) and BuChE (Asp70, Tyr332, Tyr128, Ile442, Trp82, and Glu197). Importantly, ZINC390718 demonstrated low *in vitro* toxicity in primary astrocyte-enriched glial cell cultures. The collective *in vitro* and *in silico* findings suggest that ZINC390718 holds promise as a chemotype for optimizing and identifying new dual cholinesterase inhibitors. Given its dual inhibitory activity against AChE and BuChE, coupled with low cytotoxicity, ZINC390718 emerges as a candidate meriting further investigation for the development of therapeutics targeting Alzheimer's disease [52].

Numerous studies highlight the diverse roles of butyrylcholinesterase (BChE) in various physiological and pathological contexts. In a previous investigation, efforts were directed towards gaining a precise and comprehensive understanding of mammalian BChE inhibition by the tricyclic antidepressant amitriptyline (AMI). The inquiry encompassed enzyme kinetic evaluations alongside protein-ligand docking and interaction profiling investigations. The findings supported AMI as an efficient mixed-type inhibitor of mammalian BChE, with an IC50 value of 10 μM and a Ki value of 2.25 μM. Moreover, the study provided compelling evidence that AMI effectively penetrates the active-site gorge of BChE, establishing noncovalent interactions with both choline binding and catalytic residues. These revelations hold promise for mitigating the adverse metabolic implications associated with acquired BChE deficiency and could potentially contribute to the development of innovative, reversible anticholinesterase medications in the future. [53].

In a recent study, researchers explored the inhibitory capacities of previously synthesized 9-phosphoryl 9,10 dihydroacridines and 9-phosphorylacridines against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterase

(CES). Additionally, they investigated the effects of these compounds on the self-aggregation of β-amyloid (Aβ42) using the thioflavin test and evaluated their antioxidant properties through ABTS and FRAP assays. To further elucidate the experimental findings, they employed molecular docking, molecular dynamics simulations, and quantum-chemical calculations.

The compounds from the earlier study exhibited modest inhibition of AChE and off-target CES. However, the most intriguing findings were related to the BChE inhibitory capabilities of dihydroacridines containing aryl substituents in the phosphoryl moiety, particularly the dibenzyloxy derivative 1d and its diphenethyl bioisostere 1e, which displayed the most significant activity (IC50 2.90 ± 0.23 μM and 3.22 ± 0.25 μM, respectively). Among the acridine class, only 2d, resembling the dihydroacridine 1d, emerged as an effective BChE inhibitor (IC50 6.90 ± 0.55 μM), consistent with docking predictions. Furthermore, dihydroacridines, notably 1d and 1e, demonstrated considerable inhibition of Aβ42 self-aggregation (58.9% ± 4.7% and 46.9% ± 4.2%, respectively). Overall, the most promising outcomes were identified in two dihydroacridine derivatives, 1d and 1e, distinguished by dibenzyloxy and diphenethyl substituents in the phosphoryl moiety. These compounds exhibited noteworthy inhibition of BChE activity and Aβ42 self-aggregation. These findings pave the way for further comprehensive investigations into the potential of 1d and 1e as agents against Alzheimer's disease. [54].

With the growing prevalence of Alzheimer's disease (AD), there's an urgent need for improved medication options that offer enhanced treatment outcomes with minimal side effects. Cholinesterase enzymes are pivotal in AD development, making them potential therapeutic targets. In an experiment, eight organoruthenium (II) chlorido complexes (1ah) containing pyrithione type ligands were synthesized. These ligands, ranging from 1-hydroxypyridine-2(1H) thione (a) to its methyl derivatives (b-e) and bicyclic aromatic analogs (f-h), underwent testing for their inhibitory effects on electric eel acetylcholinesterase (eeAChE) and horse serum butyrylcholinesterase (hsBuChE). The newly discovered compound 1g, incorporating the ligand 1-hydroxyquinoline-2(1H)-thione, displayed enhanced inhibition against eeAChE (IC50 4.9 μM) and even more potent action against hsBuChE (IC50 0.2 μM) compared to

the reference 1a. Computational investigations on Torpedo californica AChE corroborated these experimental findings, identifying 1g as the most energetically favorable compound and predicting precise interactions with the target protein.

This study illustrates the feasibility of selectively expanding the aromatic ring of the ligand (a) at the appropriate position as an effective strategy for enhancing activity against cholinesterases. [55].

In response to our research findings, we conducted a comprehensive evaluation to assess the impact of metal complexes, specifically cobalt (Co), nickel (Ni), and copper (Cu) complexes, on the enzymatic activity of BChE. Unlike acetylcholinesterase, BChE plays a crucial role in hydrolyzing acetylcholine within the brain. Our investigation revealed distinct inhibitory effects exerted by each metal complex on BChE, indicating the significant influence of metal properties and coordination with ligands in determining their inhibitory activity. These insights provide valuable implications for potential applications in enzyme inhibition and neurobiology. According to IC50 values, indicative of the concentration required for 50% inhibition, H2-Ni demonstrated the most effective inhibition of BChE with an IC50 of 4.53 μM, highlighting its strong inhibitory capacity. Conversely, A1-Co exhibited the lowest inhibition, with an IC50 of 34.66 μM. These specific IC50 values shed light on the differential inhibitory potency of the metal complexes, emphasizing the nuanced nature of their interactions with BChE. A comprehensive understanding, including considerations of concentration, reaction kinetics, and comparisons with existing literature, will enhance our grasp of the implications of these metal complexes in biological systems and their potential therapeutic applications.

Human pancreatic lipase (hPL) activity is critical for lipid absorption, and its inhibition can effectively reduce triglyceride intake, thus preventing and treating obesity [56]. Obesity treatment significantly decreases the prevalence of chronic metabolic diseases and mortality rates. However, the FDA-approved hPL inhibitor, Orlistat, is associated with unfavourable side effects such as diarrhea, bloating, and fecal incontinence, with incidences reaching 91%. Therefore, there is a pressing need to develop healthier and more efficient drugs focused on addressing the health risks associated with obesity.

Rapid and efficient screening approaches are essential for drug development in this context [56].

In a recent study, a high-throughput screening method for human pancreatic lipase (hPL) inhibitors was developed due to their superior anti-obesity properties and sensitivity, aiming to identify potential anti-obesity agents. A visual high-throughput assay was employed to screen hPL inhibitors using resorufin lauryl ester (RLE) as a fluorogenic substrate in a 96-well microplate. Orlistat, a specific hPL inhibitor, substantially inhibited hPL catalyzed RLE hydrolysis in a dose-dependent manner, with an IC50 value of 2.5 nM, consistent with its reported inhibitory action (IC50 6.16nM). The study evaluated the inhibitory effects of 94 commonly used clinical drugs and 94 natural products derived from Chinese medicine, revealing six strong inhibitors with significant hPL-inhibitory effects: procyanidin, carnosol, sciadopitysin, ivermectin, raloxifene, and sorafenibtosylate. The IC50 values for procyanidin, carnosol, and sciadopitysin were 0.5μM, 0.5μM, and 1.3μM, respectively, while the IC50 values for ivermectin, raloxifene, and sorafenibtosylate were 1.7μM, 5.6μM, and 15.5μM. The RLE-based assay demonstrates great potential for visual high-throughput screening of hPL inhibitors, as indicated by the results. This approach holds promise for identifying novel anti-obesity agents efficiently and effectively. [56].

In a previous study focused on evaluating the inhibitory capacities of ten flavonoid compounds against pancreatic lipase, a key target for anti-obesity agents, both in vitro and in silico methodologies were utilized to assess their efficacy. The results indicated that all examined compounds exhibited moderate inhibitory effects compared to the reference compound, orlistat. Particularly noteworthy was F01 (5-hydroxy-2-(4 hydroxyphenyl)-7-methoxychroman-4-one), which demonstrated the highest efficacy with an IC50 value of 17.68 ± 1.43 μM, acting through a competitive inhibition mechanism. Computational studies revealed that despite lacking an active functional group for covalent bonding, F01 adopted a binding mode similar to orlistat, establishing a hydrogen bond with a crucial amino acid. These findings suggest that F01 holds significant promise as a candidate for further investigations into its anti-obesity properties. This compound shows potential for development as a novel

therapeutic agent in the fight against obesity, warranting further research to explore its efficacy and safety profile. [57].

Salah Abouzied et al. conducted a study focusing on pancreatic lipase (PL) inhibition as a viable strategy for managing obesity. They designed 10 novel thiazole-benzimidazole conjugates and evaluated their potential through in silico experiments. Compounds 3, 6, and 8 were identified as having optimal drug-likeness properties and the highest binding affinity to PL. These promising derivatives were synthesized and subjected to in vitro PL inhibition assays, confirming their potency. Compound 8 displayed the highest activity, with an IC50 value of 50.09 μM, comparable to the established anti-obesity drug orlistat. The study highlights compound 8 (N-(2-((1, 2, 3-phenylallylidene) amino)-1H-benzo[d] imidazol-6-yl) thiazol-2-amine) as a highly potent PL inhibitor, suggesting its potential as a lead compound for the development of novel anti-obesity agents. The synthesized compounds underwent thorough characterization using various analytical techniques, including FTIR, 1H-NMR, 13C-NMR, and LCMS. These findings offer valuable insights into the design and development of effective treatments for obesity management. [58].

In response to our research findings, we conducted a comprehensive evaluation to assess the impact of metal complexes, specifically those composed of cobalt (Co), nickel (Ni), and copper (Cu), on the enzymatic activity of pancreatic lipase. Pancreatic lipase plays a pivotal role in hydrolysing lipids within the digestive system. Our investigation unveiled distinctive inhibitory effects exerted by each metal complex on pancreatic lipase. The observed variations in inhibition rates among the Co, Ni, and Cu metal complexes underscore the significant influence of metal properties and coordination with ligands in determining their inhibitory activity on pancreatic lipase.

These findings provide valuable insights into potential applications and mechanisms of action for these metal complexes in the field of enzyme inhibition and digestive physiology. According to the IC50 values, indicative of the concentration required for 50% inhibition, H2-Ni demonstrated the most effective inhibition of pancreatic lipase with an IC50 of 0.36 μM, highlighting its strong inhibitory capacity. In contrast, A1-Cu exhibited the lowest inhibition, with an IC50 of 26.66 μM. These specific IC50

values shed light on the inhibitory potency of the metal complexes, further emphasizing the nuanced nature of their interactions with pancreatic lipase. A comprehensive understanding, incorporating considerations of concentration, reaction kinetics, and comparisons with existing literature, will enhance our grasp of the implications of these metal complexes in biological systems and their potential therapeutic applications in digestive disorders.

REFERENCE

1 Sabale P, Patel J and Patel, "Metal Complexes: Current Trends and Future Potential", ***Int. J. Pharm. Chem. Biol. Sci.,*** 2(3): 251-265, (2012).

2 Kavitha J, Sakthikumar L, Mahalakshmy R, "Synthesis, Spectral and Biological Studies of Mixed Ligand Transition Metal Complexes of Nitroketene Dithioacetal with Ephedrine", ***Indo Am. j. pharm. sci***., 05 (01): 645-658, (2018).

3 Hariprasath k, Deepthi B, Babu I, Venkatesh P, Sharfudeen S, and Soumya V, " Metal Complexes in Drug Research - A Review", ***J. Chem. Pharm. Res***, 2(4):496-499 ,(2010).

4 Czarnek K, Terpiłowska S, and Siwicki A, "Selected Aspects of the Action of Cobalt ions in the Human Body", ***Cent. Eur. J. Immunol***., 40 (2): 236-242, 2015.

5 Mishra A, Kaushik N, Verma A, and Gupta R, "Synthesis, Characterization and Antibacterial Activity of Cobalt (III) Complexes with Pyridine amide Ligands", ***Eur J Med Chem***, 43: 2189-2196 (2008).

6 Daniel K, Harbach R, Guida W and Dou Q, "Copper Storage Diseases: Menkes, Wilson's, and Cancer", ***Front Biosci (Schol Ed)***, 9: 2652-2662, (2004).

7 Kozlevar B, and Segedin P, "Structural Analysis of a Series of Copper (II) Coordination Compounds and Correlation with their Magnetic Properties Bojan", ***Croat. Chem. Acta.,*** 81 (2) 369-379, (2008).

8 Andrejevi´c T, Aleksic I, Kljun J, Po˘ckaj M, Zlatar M, Vojnovic S, Nikodinovic-Runic J, Turel I, Djuran M, and Gli˘si´c B, "Copper (II) and Silver (I) Complexes with Dimethyl 6-(pyrazine-2-yl) pyridine-3, 4-dicarboxylate (py-2pz): the influence of the Metal ion on the Antimicrobial Potential of the Complex", ***R. Soc. Chem.***, 13, 4376–4393, (2023).

9 Rasyda Y, Rahardjo S, and Nurdiyah F, "Synthesis and Characterization Complex Nickel (II) with Diphenylamine", ***IOP Conf. Ser. Mater***. Sci. Eng., 578, (2019).

10 Tripathi l. P. and Dwivedi A, "Synthesis, Characterization and α- Glucosidase Inhibition of Some Copper, Cobalt, Nickel and Zinc Complexes with N Methylethylenediamine", ***Br J Med Med Res***, 16(6): 1-11, (2016).

11 Constable B and Housecroft C, "The Early Years of 2, 2'-Bipyridine A Ligand in Its Own Lifetime", ***Molecules***, 24:3951, (2019).

12 Osowole A, Ekennia A and Osukwe A, “Synthesis, Spectroscopic and Antibacterial Properties of Some Metal (II) Mixed Ligand Complexes of Riboflavin and 2, 2’-Bipyridine Research and Reviews”, ***J Chem***. 3(1), (2014).

13 Egbele, R.O, Ohwofosirai, A, Ugbune U. Kpomah, B, Yerima, Osakwe I, S.A and Nwajei, “Synthesis and Characterization of Mixed 2, 2-Bipyridine and penicillin G metal (II) complexes”, ***Int. j. multidiscip. curr. educ. res.***, 3(2): 23-30, (2021)**.**

14 Patadiya N, Panchal N, and Vaghela V, “A Review on Enzyme Inhibitors”, ***Int. res. j. pharm.***, 12(6), (2021).

15 Agarwal P, and Gupta R, “Alpha-amylase inhibition can treat diabetes mellitus”, ***Research and Reviews Journal of Medical and Health Sciences***, 5(4),(2016).

16 Alam S, Hasan Md , Neaz S, Hussain N, Hossain Md, and Rahman T, “ Diabetes Mellitus: Insights from Epidemiology, Biochemistry, Risk Factors, Diagnosis, Complications and Comprehensive Management”, ***Diabetology***, 21, (2021).

17 C´ orkovic´ I, Gašo-Sokacˇ D, Pichler A, Šimunovic´ J, and Kopjar M, “ Dietary Polyphenols as Natural Inhibitors of α-Amylase and α-Glucosidase”, ***Life,*** 12(11), 1692, (2022).

18 Tiwari SP, Srivastava R, Singh Cs, Shukla K, Singh RK, Singh P, Singh R, Singh NL and Sharma R, “Amylases: an Overview with Special Reference to Alph Amylase”, ***J. Biosci.***, 4 (1),1886-1901, (2015).

19 Mobini-Dehkordi M, and Afzal Javan F, “Application of alpha-amylase in Biotechnology”, ***J. Biol. Today's World***, 1(1), 39-50, (2012).

20 Savaner S, and Sohani S, “Review on Microbial α-amylase, Types & Their Industrial Application”, ***Kala Sarovar (UGC Care Group-1 Journal)***, 23(04), (2020).

21 Tripathi l. P, Dwivedi A and Mishra M, “Metal Based α-glucosidase Inhibitors: Synthesis, Characterization and α-glucosidase Inhibition Activity of Transition Metal Complexes”, ***Asian J. Med. Health.,*** 2(3), 1-14, (2017).

22 Ferreira-Vieira T, Guimaraes I, Silva F, and Ribeiro F, “Alzheimer’s disease: Targeting the Cholinergic System”, ***Curr. Neuropharmacol.***, 14,101-115, (2016).

23 Marucci G, Buccioni M, Dal Ben D, Lambertucci G , and Volpini R, “ Efficacy of acetylcholinesterase inhibitors in Alzheimer’s disease”, ***Neuropharmacology***, (2021).

24 Tian Liu T, Tian Liu X, Xi Chen Q, and Shi Y, “Lipase Inhibitors for Obesity: A Review”, ***Biomed Pharmacother.***, (2020).

25 Birari R, and Bhutani K, “Pancreatic lipase inhibitors from natural sources: unexplored potential”, ***Drug Discov. Today***, 12, 19-20, (2007).

26 Gupta S, “Role of metals in human health”, ***MOJ. bioorg. org. chem.***, 2 (5), (2018).

27 Maanvizhi S, Boppana T, Krishnan C and Arumugam G, “Metal Complexes in the Management of Diabetes Mellitus: A New Therapeutic Strategy”, ***Int J Pharm Pharm Sci***, 6 (7), (2014).

28 Bharti S, Singh S, “Metal Based Drugs: Current Use and Future Potential”, ***Pharm Lett***, 1(2), 39-51, (2009).

29 Rasheed R, Eessa H, “Synthesis of Metal Complexes Derived from Salicylidene p-Aminoacetophenone”, ***Int. J. Eng. Sci. Technol***, 30(14), (2012).

30 More P, Bhalvankar R and Patta “Synthesis and biological activity of Schiff bases of aminothiazoles”, ***J. Indian Cherm. Soc.***, 78, 474-475, (2001).

31 Sharma B and Kumar V, “Has Ferrocene Really Delivered Its Role in Accentuating the Bioactivity of Organic Scaffolds?” , ***J. Med. Chem.***,64,23, (2021)

32 Kilpin K and Dyson P, “Enzyme inhibition by metal complexes: concepts, strategies and applications”, ***R. Soc. Chem.***, 4, (2013).

33 Prejanò M, Alberto M, Russo, Toscano M and Marino, “The Effects of the Metal Ion Substitution into the Active Site of Metalloenzymes: ***A Theoretical Insight on Some Selected Cases***.

34 Tordin E, List M, Monkowius U, Schindler S and Günther K, “ Synthesis and Characterisation of Cobalt, Nickel and Copper Complexes with Tripodal 4N Ligands as Novel Catalysts For The Homogeneous Partial Oxidation of Alkanes”, ***Inorganica Chim. Acta.,*** 402, 90–96,(2013).

35 Boros E, J. Dyson, and Gasser G, “Classification of Metal-based Drugs According to Their Mechanisms of Action”, ***Chem***, 9, 6(1): 41–60, (2020).

36 Ajaz A, Ashraf Shaheen M, Ahmed M, Shahzad Munawar K, Siddique A B, Karim A, Ahmad N, Fayyaz ur Rehman M, “ Synthesis of an amantadine-based novel Schiff base and its transition metal complexes as potential ALP, α-amylase, and α-glucosidase inhibitors”, ***R. Soc. Chem***, 13, 2756-2767, (2023).

37 Garnovskii A, Kharisov B, Blanco L, Sadimenko A, Uraeva, VasilChenko I, and Garnovskii D, “ Review: Metal Complexes as Ligands”, ***J. Coord. Chem.***, 55(10), 1119−1141, (2002).

38 Sakurai H, Katoh A, Kiss T, Jakusch T, and Hattori M, “Metallo allixinate complexes with anti-diabetic and anti-metabolic syndrome activities”, ***Metallomics,*** 2, 670–682, (2010).

39 Ramsay R, and Tipton K, “Assessment of Enzyme Inhibition: A Review with Examples from the Development of Monoamine Oxidase and Cholinesterase Inhibitory Drugs”, ***Molecules,*** 22, 1192, (2017).

40 Ravikumar K, Sridhar B, Aparna P and Rao J, "A polymorph of bavachalcone", ***Acta Crystallogr.***, 61, 1020-1022, (2005).

41 Feng Y, Nan H, Zhou H, Xi P, and Li B, " Mechanism of inhibition of α-glucosidase activity by bavachalcone", ***School of Food Science, Henan Institute of Science and Technology, Xinxiang, China***, 42, (2022).

42 El-shora H, Messgo S, Ibrahim M, Alfakharany M, "Induction, immobilization, modificationand natural inhibitorsofα-glucosidasefrom Penicillumchrysogenum", ***Int. J Phytomed.***, 10(4), 208, 214, (2018).

43 Sohrabi M, Binaeizadeh M, Iraji A, Larijani B, Saeedi M, Mahdavi M, " A review on a-glucosidase inhibitory activity of first row transition metal complexes: a futuristic strategy for treatment of type 2 diabetes", ***R. Soc. Chem***, 12,(2022).

44 Hichri F, Omri A, Saad Mana Hossan A, and Ben Jannet H, "Alpha-glucosidase and amylase inhibitory effects of Eruca vesicaria subsp. longirostris essential oils: synthesis of new1, 2, 4-triazole-thiol derivatives and 1, 3, 4-thiadiazole with potential inhibitory activity", ***Pharm. Biol.***, 57(1), 564-570, (2019).

45 Alqahtani A, Hidayathulla S, TabishRehman M, .ElGamal A, Al-Massarani S, Razmovski-Naumovski V, S.Alqahtani M, El Dib R, and AlAjmi M, " Alpha-AmylaseandAlpha-GlucosidaseEnzyme Inhibition and Antioxidan tPotential of 3-Oxolupenal and Katononic Acid Isolated from Nuxiaoppositifolia" ***Biomolecules***, 10,61,(2020).

46 Chen Z, Huang J, Yang S, and fang hong F, " Role of Cholinergic Signalingin Alzheimer's Disease" ***Molecules,*** 27,1816, (2022).

47 Dizdar M, Vidic D, Požgan F, Štefane B, and Maksimovi´c M, " Acetylcholinesterase Inhibition and Antioxidant Activity of N-trans-Caffeoyldopamine and N-trans-Feruloyldopamine" ***Sci. Pharm.***, 86,11.

48 Stellenboom N, "Inhibition of Carbonic Anhydrase, Acetylcholinesterase and Butyrylcholinesterase by BisPMB, A Synthetic Analogue of Ajoene", ***J. Turkish chem. soc. sect. chem.*** 6(2), 143-148, (2019).

49 Ajayeoba T, Folorunso Akinyele O, Olaolu Ayeni A, and Julius Olawuni I, " Synthesis, Characterisation and Acetylcholinesterase Inhibition Activity of Nickel(II) and Copper(II) Complexes of 3-Hydroxybenzaldehyde-4-nitrobenzoic Acid Hydrazone", ***Am. J. Appl. Chem.***,7(2): 64-71, (2019).

50 Budryn G, Majak I, Grzelczyk J, Szwajgier D, Rodríguez-Martínez A and Pérez-Sánchez, "Hydroxybenzoic Acids as Acetylcholinesterase Inhibitors: Calorimetric and Docking Sim tion Studies", ***Nutr.***, 14, (2022).

51 Mushtaq G, Greig N, A. Khan J, and A. Kamal M, "Status of Acetylcholinesterase and Butyrylcholinesterase in Alzheimer's Disease and Type 2 Diabetes Mellitus", ***CNS Neurol Disord Drug Targets***, 13 (8), 1432-1439,(2014).

52 B. M. de Almeida R, B. Barbosa D, R. do Bomfim M, A. O. Amparo J, S. Andrade B, L. Costa S, M. Campos J, N. Cruz J, B. R. Santos C, H. A. Leite F, and B. Botura M, " Identification of a Novel Dual Inhibitor of Acetylcholinesterase and Butyrylcholinesterase: In Vitro and In Silico Studies", ***Pharm.***, 16,95, (2023).

53 Teralı K, Dalmizrak O, Muhammad Uzairu S, and Ozer N, "New insights into the interaction between mammalian butyrylcholinesterase and amitriptyline: a combined experimental and computational approach", ***Turk. Biochem. Soc***, (2018).

54 Makhaeva G, V. Kovaleva N, V. Rudakova E, P. Boltneva N, V. Lushchekina S, Yu Astakhova T, N. Timokhina E, G. Serebryakova O, V. Shchepochkin A, A. Averkov M, A. Utepova I, S. Demina N, V. Radchenko E, A. Palyulin V, P. Fisenko V, O. Bachurin S, N. Chupakhin O, N. Charushin V, J. Richardson R, " Derivatives of 9-phosphorylated acridine as butyrylcholinesterase inhibitors with antioxidant activity and the ability to inhibit β-amyloid self-aggregation: potential therapeutic agents for Alzheimer's disease", ***Front. pharmacol.***, (2023).

55 Kladnik J, Ristovski S, Kljun J, Defant A, Mancini I, Sepˇci´c K, Turel I , " Structural Isomerism and Enhanced Lipophilicity of Pyrithione Ligands of Organoruthenium(II) Complexes Increase Inhibition on AChE and BuChE", ***Int. J. Mol. Sci.***, 21, (2020).

56 Bin Hou F, Zhang N, Hao Zhu G, Fan Fan Y, Ru Sun M, Liang Nie L, Bo Ge G, Juan Zheng Y, and Ping Wang, " Functional Imaging and Inhibitor Screening of Human Pancreatic Lipase by a Resorufin-Based Fluorescent Probe", ***Biosensors*** , 13, 283, (2023).

57 Tran T, Tan Mai T, Trang Ho T, Dung Le T, Nhung Cao T, Minh Thai K, and Son Tran T," Pancreatic Lipase by Flavonoid Derivatives: In Vitro and In Silico Investigations", ***Adv. Pharmacol. Pharm. Sci.***, (2024).

58 Salah Abouzied A, Khaled Bin Break M, Huwaimel B, Hussein W, Alafnan A, and Mahmoud Younes K, " Discovery of A Novel Synthetic Thiazole-benzimidazole Conjugate that Acts as a Potent Pancreatic Lipase Inhibitor using in silico and in vitro Approaches", ***Indian J Pharm Educ Res.,*** 57 (1), 218-227, (2023).

RESUME

Raneem Mamoun ALI NOUR completed her primary and secondary education at Al-Faisaliah Private School in Saudi Arabia. Throughout her academic journey, she demonstrated exceptional dedication, a strong work ethic, and an impressive ability to quickly grasp complex concepts. Her independence and technical proficiency set her apart as a student.

In 2013, she pursued higher education at the University of Petra in Amman, Jordan, where she graduated from the Nutrition Department within the Faculty of Pharmacy and Medical Sciences. After obtaining her degree, she worked as a Nutritionist with Al Nahdi Pharmacy, focusing on Illuma milk from Nestlé, and also collaborated with Tawuniya Insurance Company between 2017 and 2018. In 2020, she decided to advance her expertise by enrolling in a Master's program. She graduated in 2024 with an MSc degree in Food Toxicology from Karabük University, achieving an excellent GPA. Her academic and professional accomplishments reflect her commitment to the field of nutrition and food toxicology.