A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

INVESTIGATION OF EFFECTS OF Cu AND Pd COMPLEXES OF SOME SCHIFF-BASES ON XANTHINE OXIDASE ACTIVITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

BURAQ JASIM MOHAMMED ALAMURAD

ÇANKIRI

2023

INVESTIGATION OF EFFECTS OF Cu AND Pd COMPLEXES OF SOME SCHIFF-BASES ON XANTHINE OXIDASE ACTIVITY

By Buraq Jasim Mohammed ALAMURAD

April 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor **:** Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor **:** Asst. Prof. Dr. Ravi RAWAT

Examining Committee Members:

Chairman **:** Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Member **:** Prof. Dr. Volkan EYÜPOĞLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. Hamit ALYAR
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Buraq Jasim Mohammed ALAMURAD

ABSTRACT

INVESTIGATION OF EFFECTS OF Cu AND Pd COMPLEXES OF SOME SCHIFF-BASES ON XANTHINE OXIDASE ACTIVITY

Buraq Jasim Mohammed ALAMURAD
Master of Science in Chemistry
Advisor: Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor: Asst. Prof. Dr. Ravi RAWAT
April 2023

The metabolism of purines produces hypoxanthine, and by the action of the enzyme xanthine oxidase, it converts to xanthine, and also the enzyme (xo) converts xanthine to uric acid. If the uric acid in the blood exceeds the normal level, the kidneys cannot completely excrete it through the urine (Hyperuricemia), so it is deposited in the form of small stones in the kidneys and joints, especially the big toe (gaot), causing swelling and pain in these areas. In this study, chemical complexes containing copper and palladium were formed at different concentrations to inhibit the xanthine oxidase enzyme. Six readings were taken for each complex from the spectrophotometric device. It was found that each compound has the potential to inhibit at certain concentrations. The treatment used for gout disease is allopurinol, but this treatment has side effects such as rash and others, so this thesis aims to find an alternative drug that is more efficient and less risky than previous treatments.

2023, 49 pages

Keywords: Xanthine oxidase inhibitor, Allopurinol, Gout, Uric acid, Hyperuricemia

ÖZET

SCHIFF-BASE (Cu VE Pd) GEÇİŞ METAL KOMPLEKSLERİYLE KSANTAN OKSİDAZ ENGELLENİR VE ÜRİK ASİT SEVİYELERİ DÜŞÜRÜLÜR

Buraq Jasim Mohammed ALAMURAD
Kimya, Yüksek Lisans
Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU
Eş Danışman: Dr. Öğr. Üye. Ravi RAWAT
Nisan 2023

Pürinlerin metabolizması hipoksantin üretir ve ksantin oksidaz enziminin etkisiyle ksantine dönüşür ve ayrıca (xo) enzimi ksantini ürik aside dönüştürür. Kandaki ürik asit normal düzeyi aşarsa böbrekler bunu tamamen idrarla dışarı atamaz (Hiperürisemi), bu nedenle böbreklerde ve eklemlerde, özellikle ayak başparmağında (gaot) küçük taşlar şeklinde birikerek ürik asit oluşumuna neden olur. Bu bölgelerde şişlik ve ağrı. Bu çalışmada, ksantin oksidaz enzimini inhibe etmek için farklı konsantrasyonlarda bakır ve paladyum içeren kimyasal kompleksler oluşturulmuştur. Spektrofotometrik cihazdan her kompleks için altı okuma alındı. Her bileşiğin belirli konsantrasyonlarda inhibe etme potansiyeline sahip olduğu bulundu. Gut hastalığı için kullanılan tedavi allopurinol'dür, ancak bu tedavinin kızarıklık ve diğerleri gibi yan etkileri vardır, bu nedenle bu tez önceki tedavilere göre daha etkili ve daha az riskli alternatif bir ilaç bulmayı amaçlamaktadır.

2023, 49 sayfa

Anahtar Kelimeler: Ksantin oksidaz inhibitörü, Allopurinol, Gut, Ürik asit, Hiperürisemi

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU, for his patience, guidance and understanding.

I would also like to dedicate this humble effort to my loving mother, my older brother, and everyone who helped me.

Buraq Jasim Mohammed ALAMURAD
Çankırı-2023

CONTENTS

LIST OF SYMBOLS

μL	Microliter
M	Molarity
mg	Milligram
mL	Milliliter
mmol	Millimole

LIST OF ABBREVIATIONS

AMP	Adenosine monophodphate
COPD	Chronic obstructive lung disease
GMP	Guanine monophosphate
IMP	Inosine monophodphate
MSU	Monosodium urate
ROS	Reactive oxygen species
SUA	Serum uric acid
XO	Xanthine oxidase

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The liver and intestine are where xanthine oxidase, a protein enzyme, is most active. In the digestive system, XO is heavily concentrated. In order to create uric acid from hypoxanthine, the enzyme xanthine oxidase must first hydroxylate xanthine. Uric acid plasma levels increase naturally with age; they are lower in adult women and are equal to those of males in postmenopausal women (Burini 2012). A rise in reactive oxygen species (ROS) level is taken into account during the reaction process. As a result, XO is a significant generator of uric acid and ROS. The key to gout disease is hyperuricemia, Additionally, the development of hyperlipidaemia, hypertensions, , obesity and diabetes they everyone were associated to the increased risk of hyperuricemia (Ikram *et al.* 2015), which is brought on by an excessive production of uric acid. Increased levels of xanthine oxidase can result in oxidative stresses, mutagenesises, and even cancer. As a result, XO inhibition reduces oxidative stress just after inflammation. In addition, blocking XO might be a technique to treat cancer (Türker *et al.* 2020). Production of reactive oxygen species (RSO) by XO has become necessary to inhibit this enzyme, so there were many drugs that reduce the activity of xanthine enzyme, especially allopurinol, but it remains weak towards eliminating reactive oxygen species and also because of its side effects prompted many researchers On the discovery of a new drug that has the ability to inhibition of the xanthine enzyme more, also with few side effects, so studies relied on mineral complexes for this purpose (Hadizadeh *et al.* 2009). The liver readily absorbs purine bases and their nucleotides and transformed into xanthines, degraded uric acid, which is recycled via the salvage pathways, or synthesized from scratch, however the latter needs more energy (ATP consumption). By the action of xanthine oxidase, xanthine produces uric acid via hypoxanthine. Uricase transforms uric acid in the majority of mammals into allantoin, which is then turned into urea and expelled. This is the cause (Burini 2012). Diseases including leukemia, leukocytosis, and dystrophies that cause significant cell loss potentially enhance the liver's intake of nucleic acids and the production of uric acid. As a result, disorders brought on by inherent purine metabolic mistakes may also cause hyperuricemia.

In the human body, reactive oxygen species (ROS), which are oxygen-based free radicals, are extensively dispersed and damage cells, causing type two diabetes and gout. The oxidation of numerous cell membrane lipids in type two diabetes has been linked to free radicals. As a result of this process, islet cells' ability to release, produce, or otherwise absorb ingredients for insulin is interfered with. Similarly, the low-density lipoprotein (LDL) molecule's ability to oxidize the lipid cholesterol, results in atherosclerosis, another extremely dangerous illness. Oxidized lipids have also been linked to some other degenerative disorders, such as gout, cardiovascular risk factors, damage to the corneal epithelium and endothelium, chronic obstructive pulmonary disease (COPD), and renal failure brought on by the formation of uric acid crystals, and other conditions are all significantly impacted by oxidase. Additionally, XO can create reactive nitrogen species, such as peroxynitrite, which has been demonstrated to interact with DNA, proteins, and cells in a way that is harmful or poisonous.. It has been discovered that reactive nitrogen signaling and ROS play a role in myocardial and vascular functioning. Gabapentin and 2-hydroxynaphthaldehyde combined to form a brand-new Schiff base molecule. The ability of the -OH substituted Schiff bases to scavenge free radicals has previously been examined. Because of their extraordinary capacity to coordinate, including the -C=N- linkage electron system, Schiff bases are highly utilized as ligands. When these Schiff bases with chiral moiety are complexed, a geometrical constriction is created; this characteristic is used to increase the catalytic activity of oxidation for transition metal complexes. Complex formation may be accompanied by modifications in magnetic and electrochemical behavior, which can be fine-tuned to improve a variety of applications, such as catalysis, pharmaceutical use, and laboratory reagents. In order to coordinate metals, monoanionic and bidentate ligands based on salicylaldehyde imiminium were used. Studies looked at whether or not transition metal complexes with such bidentate ligands have enhanced dioxygen reactivity. The reactivity of various metal complexes to dioxygen or free radicals was expected to disclose the interaction patterns inside the body because Schiff base ligands were considered to be possible models for the active areas of proteins. It's interesting to note that zinc-based supplements or substances have been shown to reduce xanthine oxidase activity quite some time ago. The development of new diseases and treatments for conditions caused by xanthine oxidase activity and reactive oxygen species ,

,therefore seemed to be a particularly promising goal when combining all the factors outlined above. Here, we report compounds of the newly discovered Gabapentin, which carries a Schiff base ligand, with divalent metal ions like Pd, Cu, and Zn (Li *et al.* 2017). Their capacity to quench DPPH free radicals and inhibit xanthine oxidase was evaluated.

2. LITERATURE REVIEW

Protein compounds called enzymes play an important role in stimulating and accelerating chemical processes inside the body's most critical cells. Enzymes enter reactions in small quantities without any change in their chemical composition. The enzyme performs its function completely under physiological conditions such as temperature, pH, and substrate (Wood 1996).

2.1 Nomenclature and Classification of Enzymes

Enzymes are essential for maintaining the life processes of living things since they regulate various chemical processes like metabolism, nutrition, energy conversion, and many others that are required for the survival of the living thing's cells. Most enzymes are composed of proteins, but some of them are composed of ribonucleic acid (Tao *et al.* 2020), since the main substance in the enzyme is protein. In order to categorize enzymes using computational models and to make enzyme research easier, proteins are always used as the basis for extracting features based on them. The Group for Enzymes, a global committee created by the International Union for Biochemistry (IUB), is in charge of creating the language for enzymes (Table 2.1). The committee divided enzymes into seven main four pieces of figures make up the EC (Enzyme Commission number) nomenclature, which identifies the main class of the enzyme, its subclasses, its sub-subclasses, and its class of substrates. Enzymes may be categorized using the majority of academic disciplines down to the subclass level categories (Cheng *et al.* 2012).

Table 2.1 Enzyme classifications and subcategories

Main classes	***Subclasses***
Oxidoreductases EC1	Enable oxidations and reductions to occur.
Transferases EC2	Enable the transfer of molecules like phosphoryl, methyl, or glycosyl groups.
Hydrolases EC3	accelerate the hydrolytic breakage of other bonds, such as C—C, C—O, and C—N.
Lyases EC4	by catalyzing the removal of atoms from other bonds, such as C—C, C—O, and C—N, double bonds are left behind.
İsomerases EC5	cause modifications in a molecule's geometry or structure.
Ligases EC6	catalyze the fusion of two molecules in conjunction with the hydrolysis of ATP.
Ttanslocases EC7	Translocation of a proton, translocation of amino acids, translocation of cation, and translocation of the anion.

2.2 Control Over the Metabolism and Uric Acid Excretion

There are a variety of factors that control the intricate processes of uric acid formation in the liver, and its subsequent excretion through the kidneys and intestines. Uric acid is produced when the body processes both endogenous and exogenous purines. The external This purine pool, which is mostly contributed by animal proteins and fluctuates significantly with diet. The inborn uric acid production the primary suppliers of acid include the liver, intestines, and various tissues, including muscles (Chaudhary *et al.* 2013). The molecular weight of uric acid, a C5H4N4O3 (7, 9-dihydro-1H-purine-2, 6, 8(3H)-trione) heterocyclic organic molecule, is 168 Da. The two-purine nucleosides are converted by a variety of enzymes. To uric acid are converted the nucleic acids adenine and guanine. Adenosine monophosphate (AMP) can be converted into inosine in two different ways: either by first removing an amino group by deaminase to form inosine monophosphate (IMP), then by ephosphorylation with nucleotidase to form inosine, or by first removing a phosphate group by nucleotidase to form adenosine, then by deamination. The chemical name for this molecule is guanine monophosphate, or guanine for short. As a result of the action of the enzyme nucleotidase, guanosine is altered. In addition, purine nucleoside phosphorylase changes inosine and guanosine into hypoxanthine and guanine, respectively, both of which are purine bases (Maiuolo *et al.* 2016). Xanthine-oxidase (XO) converts hypoxanthine to xanthine, while guanine deaminase converts guanine to xanthine. Again oxidizing xanthine with xanthine oxidase yields uric acid. It's enzymes like these that break down purines, and they're mapped out in (Figure 2.1). Uric acid has a pKa of 5.8, making it a weak acid at neutral pH. The acid's salt, urate, is the most often consumed form of uric acid. The development of uric acid crystals rises in tandem with the concentration of urate in the blood. In men, the normal range for uric acid in the blood is between 2.5 and 7.0 mg/dL, whereas in women, it is between 1.5 and 6.0 mg/dL. Although uric acid is not particularly soluble in water, the usual human blood uric acid content is quite close to the solubility limit (6.8 mg/dL). If your uric acid level is above 6.8 mg/dL, you may develop crystals of monosodium urate, which are uric acid (MSU). Uric acid cannot be converted to the more soluble molecule allantois in humans due to a lack of the uricase enzyme. Urine is the primary route of uric acid elimination on a daily basis (Jin *et al.* 2012). Reportedly, anomalies in the transport of urate are the consequence of dysfunctions in the three urate transporters URAT1/SLC22A12, GLUT9/SLC2A9, and ABCG2/BCRP. Among them, it has been shown that widespread ABCG2 exporter dysfunction is a major contributor to hyperuricemia and gout. In addition, renal hyperuricemia is exacerbated by increased urate excretion (Matsuo *et al.* 2014). Among the family of glucose transporters (GLUT or SLC2), GLUT9 (SLC2A9) is distinguished by its substrate selectivity and sequence identity. It was shown that GLUT9 mostly transports urate, even while the other 13 members of the GLUT superfamily transport glucose or other monosaccharides. Single nucleotide polymorphisms in the SLC2A9 genes have also been associated with gout, coronary artery disease, and myocardial infarction. All 14 members of the GLUT family share structural features such as twelve transmembrane helices, amino and carboxyl termini in the cytoplasm, and an N-linked glycosylation site. However, each member of the family has a different glycosylation site. SLC2A9a and SLC2A9b are two isoforms that encode hGLUT9a and b, respectively, and differ only in the first 29 residues of their N-terminal domains. While GLUT9a is expressed in most tissues, GLUT9b is restricted to the principal urate-transporting organs (liver and kidney). Research on GLUT9-mediated uric acid transport has been conducted. In spite of the fact that it does not need anions, sodium, or chloride, it is voltage-dependent, and currents have been found to Without excluding alternative possibilities, the data thus far is most consistent with a transport model in which GLUT9 is a uniport (Clémençon *et al.* 2014).

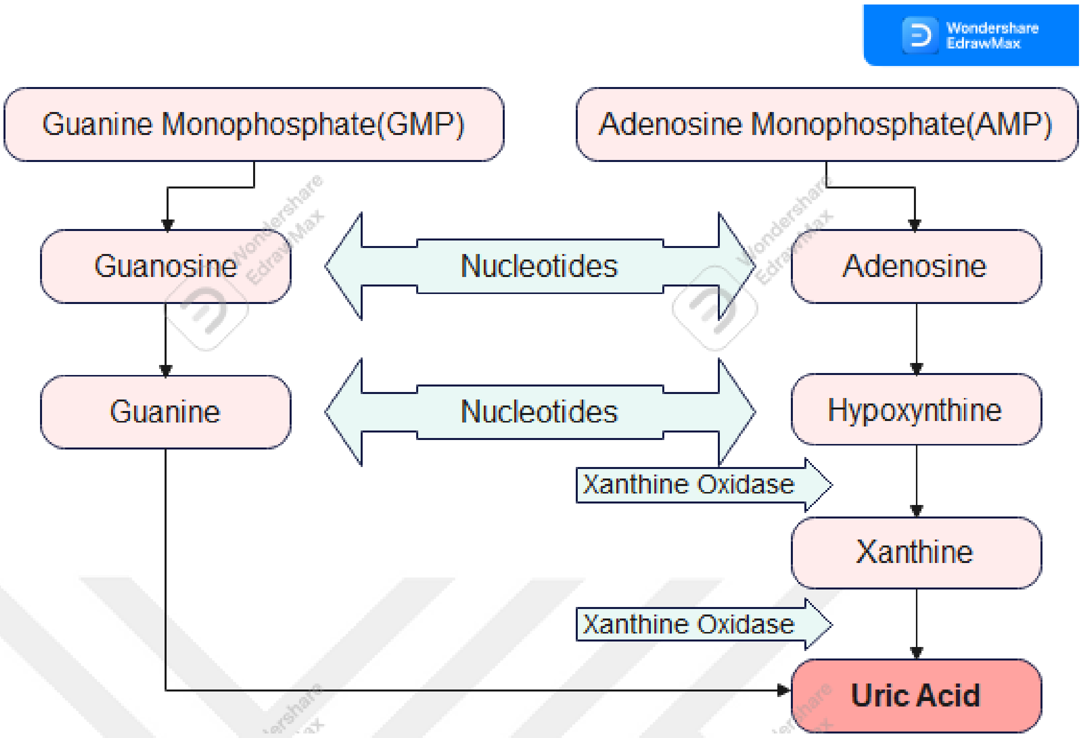


Figure 2.1 Humans have enzymes that break down purines (Maiuolo *et al.* 2016)

2.3 The Relationship between Reactive Oxygen Species and Enzymes

Figure 2.2 shows that reactive oxygen species (ROS) such hydrogen peroxide, superoxide radical anion, hydroxyl radical, alkyl peroxyl radical, nitric oxide, and singlet oxygen are often associated to many physiopathological diseases in humans. Oxidative stress, caused by an imbalance between antioxidant systems and the production of oxidants, including ROS (Johar *et al.* 2006, Halliwell 1989), is thought to play a role in the etiology of a variety of diseases, including arthritis, cancer, and inflammation. Other degenerative conditions and processes that may be affected by oxidative stress include atherosclerosis, Parkinson's disease, Alzheimer's dementia, and reperfusion injury of the brain or heart. The human body produces reactive oxygen species (ROS) when exposed to various exogenous chemicals in the environment, such as ultraviolet (UV) light, X-rays, and gamma rays, or when processes are accelerated by metals. The human body generates reactive oxygen species (ROS) through a variety of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer and through exposure to a wide variety of exogenous chemicals in our environment, such as those generated during metal-catalyzed reactions, UV light irradiation, X-ray exposure, and gamma radiation. Byproducts of electron transport mechanisms mediated by mitochondria, such as those that occur during inflammation, might include ROS. Endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer, as well as exposure to numerous exogenous chemicals in our environment, such as those produced during metal-catalyzed reactions, UV light irradiation, X-ray exposure, and gamma radiation, all contribute to the production of ROS in the human body (Cadenas 1989, Johar *et al.* 2006). When there is inflammation, neutrophils and macrophages can produce ROS as byproducts of electron transport processes that are mediated by mitochondria, among other endogenous causes (Kostić *et al.* 2015). The enzyme xanthine oxidase, which converts hypoxanthine to xanthine and xanthine to uric acid, is a further biological source of superoxide radicals. Mitochondria, cytochrome P450 metabolism, peroxisomes, and the activation of inflammatory cells are all endogenous producers of superoxide radicals (Cadenas and Davies 2000). Molecular oxygen is reduced in both methods, first producing the superoxide anion and later the hydrogen peroxide (Valko *et al.* 2004). The importance of exogenous antioxidants, especially those obtained through food, in lowering oxidative stress has also been proven by a number of researches. The vast majority of these antioxidants are phenolic chemicals, such as flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, and tocotrienols (Laguerre *et al.* 2007).

$$\text{Hypoxanthine} + O_2 + H_2O \longrightarrow \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{UA} + O_2^- + H^+$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{UA} + H_2O_2$$

$$O_2^- + H^+ \longrightarrow H_2O_2 + O_2$$

Figure 2.2 Hydrogen peroxide formation

2.4 Xanthine Oxidase

Xanthine oxidase (XO; EC 1.17.3.2) is a multifunctional molybdoflavoprotein that is extensively distributed and found in the vascular endothelium, kidney, lung, and milk. The liver and gut have the greatest specific XO activity in humans (Harrison 2002). The enzyme is crucial in the development of hyperuricemia and gout, catalyzing the conversion of hypoxanthine and xanthine to uric acid as part of the metabolism of purines. Under physiologically normal circumstances, XO is present as xanthine dehydrogenase (XDH), which is transformed into XO by the oxidation of sulfhydryl residues or by res tricted proteolysis (Tsujii and Nishino 2008). Superoxide anions and H2O2 are produced by XO during the catalytic oxidative hydroxylation of purine substrates, and in the presence of chelated iron, they are transformed into very reactive hydroxyl radicals (Stockert *et al.* 2002). The pathogenic processes that these reactive oxygen species (ROS) are linked to include inflammation, metabolic disorders, cellular aging, reperfusion injury, atherosclerosis, and carcinogenesis. ROS trigger cell signaling cascades, promote or decrease the expression of numerous genes, and cause necrosis or programmed cell death. Additionally connected to ROS are the oxidative damage to lipids and DNA that contribute to the emergence and progression of vascular disease. Many illnesses, including gout, or at least signs of diseases, such as oxidative damage to the tissues, are caused by the excess level of metabolites of XO, such as uric acid and ROS (Pacher *et al.* 2006). A broad range therapy for gout, cancer, inflammation, and oxidative damage may thus come from the specific suppres\ion of XO.

2.5 Xanthine Oxiadse Inhibitation

In many disease cases, it is necessary to inhibit or stimulate some types of enzymes to get rid of the risks of that disease, for example in this study is the enzyme xanthine, where it is necessary to inhibit it in some cases, as mentioned previously (Wood 1996). First, there is competitive inhibition, in which the substrate and the inhibitor both try to occupy the enzyme's active site. This is one of four distinct forms of inhibition. This process does not rely on binding to the enzyme's active site, but rather to allostric sites. The third type is uncompetitive inhibition that binds to the enzyme that binds to the substrate but the inhibitor binds to the enzyme from the allostric site. The last type is irreversible inhibition, in which the inhibitory factor binds to the enzyme and causes structural changes in the stereotyped permanently and irreversibly, permanently irreversibly inhibiting the action of the enzyme.

2.5.1 Inhibition by allopurinol

Though first developed as an anticancer, allopurinol was later shown to have uric acid-lowering effects via inhibiting the enzyme xanthine oxidase. Allopurinol has been used to treat gout and hyperuricemia since it was given the green light by the FDA in 1966 (Van Peenen and Joseph 1975). It is the enzyme xanthine oxidase in living organisms that quickly oxidizes allopurinol to oxypurinol, which then inhibits xanthine oxidase. Allopurinol is an example of a competitive inhibitor at low doses but a non-competitive inhibitor at high quantities. The prolonged tissue retention and subsequent production of allopurinol are the primary mechanisms responsible for its pharmacological action. In terms of the pharmacokinetics of allopurinol, it is rapidly absorbed and reaches the plasma from 30 to 60 minutes.

Gastrointestinal discomfort, hypersensitivity responses, and rash are the most common side effects of allopurinol. Even after taking the drug for months or years, an anaphylactic reaction may persist. People with impaired renal function, for whom the dose of allopurinol has not been reduced, usually experience these side effects. Allopurinol reduces the metabolism of oral coagulants and probenecid and may increase the effects of cyclophosphamide. Fever, rash, vasculitis, eosinophilia, and decreased renal function are signs of allopurinol toxicity, which can be fatal, especially in elderly patients with renal failure (Rouquette *et al.* 1998).

Crystals in the kidneys may be broken up with the help of allopurinol. Helps prevent arthritis from becoming chronic. As uric acid levels drop during therapy, kidney stones dissolve and the patient's health is protected from further damage. There are many therapeutic possibilities of allopurinol and in various forms in brain tissue and other types of tissues, vascular injuries, inflammatory diseases and heart failure (Pacher *et al.* 2006).

2.5.2 Inhibition by schiff-base transition metal

There are several documented Schiff base transition metal complexes to possess antibacterial and anticancer properties. It was disclosed that when taken, certain medications have increased activity compared to those of free organic molecules as metal complexes. So, There may be latent potential in Schiff base transition metal complexes container for medications (You *et al.* 2008). Schiff bases with an azomethine group (C=N) are of great interest due to the fact that the sp2-hybridized orbital of the nitrogen atom in the azomethine group correlates with the number of transition metal ions. Among the many areas in which they excel is as essential intermediates in organic synthesis, as well as in coordination chemistry, industrial biology, pharmacology, catalysis, and optics. A hydroxyl group (OH) in a schiff base gives it unique properties in chemistry and physics, including photochromic and thermochromic properties. Schiff base ligands derived from salicylaldehyde and its derivatives play a crucial role in the production of metal complexes, which has several applications in bioinorganic chemistry, catalysis, and magnetochemistry. Several Schiff base complexes with metals have attracted a lot of investigation due to their diverse biological and pharmacological effects, such as their anticancer, antioxidative, antiviral, antibacterial, and antineoplastic capabilities (Buldurun *et al.* 2020). The product of purine metabolism, especially superoxidized anion, which causes ischeamia tissue injury, edema, and vascular permeability, which led to the production of many xanthine oxidase inhibitors, such as the strong inhibitor allopurinol, but Schiff bases were rarely used for this purpose It has a high potential for inhibition with few side effects (Buldurun *et al.* 2020).

2.5.3 Inhibition by flavonoids

Antioxidant, antibacterial, antiviral and antimutagenic activities are only some of its well-known biological and pharmacological effects. Several enzymes, including cyclooxygenase, lipooxygenase, and phosphoinositide 3-kinase, have been demonstrated to be potently inhibited by them. Gout is brought on by xanthine oxidase, which also causes oxidative injury to living tissues. The homodimer of xanthine oxidase with a molecular weight of 290Kd is the active form, and each monomer independently catalyzes the activity. One FAD cofactor, two [2Fe-2S] centers, and one molybdopterin cofactor are present in each subunit, originally disclosed the salicylate-xanthine oxidase complex co-crystal structure. Substrate binding and oxidation occur at the molybdopterin core (Mo-pt). It was previously understood that salicylate acted as a competitive inhibitor by binding to the Mo active site. Although salicylate does not directly bind to the Mo-pt cofactor, it nonetheless blocks substrate access to the metal complex. Numerous amino acid residues, including Arg 880, Phe 914, Phe 1009, Thr 1010, and Glu 1261, contributed to salicylate and the binding site's essential hydrogen and electrostatic interactions. Xanthine oxidase is an enzyme that flavonoids have been demonstrated to block. Furthermore, flavonoids that have been shown to interact with this enzyme have had their structure-function relationship investigated (Hofman *et al.* 2007).

2.6 Effect of Oxidation with Xanthine Oxidase Enzyme

Many physiological processes related to normal and abnormal bodily functioning include the superoxide radical ion. It has long been known that the superoxide radical O2- plays an essential role in both healthy biological processes and the development of illness. It is common for immunological defense cells and many other kinds of accessory cells to generate extracellular superoxide radicals. For all living things, the production of O2 is a given, since it plays a role in many physiological processes, including the development of cancerous cells and the spread of tumors (Burdon 1995). Superoxide's significance in the onset and progression of cancer is intricate.

By shifting the redox balance, oxidative stress and the unchecked buildup of metabolic free radicals caused by a shortage of reducing agents, both of which contribute to carcinogenesis, may be used to kill off cancerous cells.

Xanthine oxidase is among the most significant enzymes in terms of the amount of superoxide it generates. It is a member of the molybdenum-containing protein family. There are two separate groupings within it. Two substrate-binding sites are found in each class. Each fork has molybdenum, iron, and sulfur naproxen centers, as well as Flavin adenine dinucleotide (FAD) to convert xanthine to uric acid. During xanthine oxidase re-oxidation, molecular oxygen serves as an electron acceptor, leading to the formation of superoxide and hydrogen peroxide. As a result, any change in xanthine oxidase activity may have an impact on the body's ability to keep its cellular levels of superoxide, a potentially harmful byproduct of normal cellular metabolism, under control (Burdon 1995).

In the quest for a way to effectively eradicate cancerous cells, a number of strategies have been developed to regulate the superoxide generated by xanthine oxidase. Cancer cells are the target of chemotherapy, which works by triggering their programmed death. The use of many medications that raise the tumor's oxidative stress levels accomplishes this goal. Having qualities typical of pro-oxidants, such as the promotion of metal ions included in chemical complexes, the induction of membrane permeability and apoptosis, the elevation of oxidative damage caused by reactive oxygen species, and the modification of cytoskeleton assembly and stability (Kostova 2005). Given that the transport of electrons to the tumor is inhibited by cancer, these properties make them promising pharmacological agents in the treatment of cancer. To successfully and scientifically circumvent its damaging impact on healthy cells, the need for the discovery of novel complex chemicals with enhanced anticancer activity and their protective effect on normal cells may arise. Complexes containing highly reactive cations surrounded by physiologically active links with antioxidant capacity have been proposed as a potential remedy. This substance maintains its stability at a pH of 7 (Todorov *et al.* 2021).

3. MATERIALS AND METHODS

3.1 Materisls

3.1.1 Regents

Phosphate buffer (0.5 M) (pH7.5), Hypoxanthine and xanthine.

3.1.2 Equipment

The following equipment and tools were utilized during the studies:

Mixer (Vortex)	:	Fisons whirlmixer
pH meter	:	Sehott pH-Meter CG840
Precision balance	:	Late Avery
Automatic pipette	:	Eppendorf
Magnetic stirrer	:	Chilten Hot Plate Magnetic Stirrer ACCOUNT
Distill water device	:	Barnstead Easy Pure UV/UF
Refrigerators	:	Boch
Deep freeze	:	Sanyo Medical Freezer
Multiplate Reader	:	

3.1.3 Solution used and their preparation

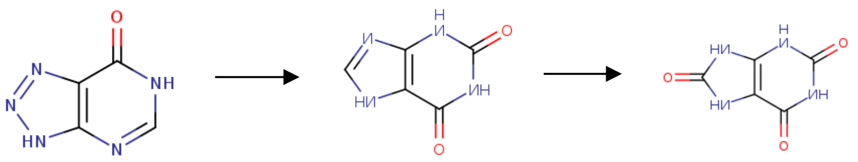
1- 0.5 M of phosphate buffer (pH=7.5) dissolve 11.931 g of sodium hydrogen phosphate in water and dilute to 100 ml distelled water (solvent A). Dissolve 4.536 g of potassium dihydrogen phosphate in distelled water and dilute to 100 ml (solvent B). Mix 85 ml of solvent A with 15 ml of solvent B.

2- Weigh 4 milligrams of hypoxanthine and dissolve it in 9ml of distilled water and 1ml buffer.

3- Xanthine oxidase enzyme in different quantities.

3.2 Methods

3.2.1 Measurement of enzyme activity



Hypoxanthine Xanthine Uric acid

From the previous equation, the enzyme xanthine oxidase increases the production of uric acid by relying on two compounds, hypoxanthine and xanthine, so it is necessary to inhibit the xanthine oxidase enzyme to reduce the formation of uric acid. This is done through an enzymatic reaction to remove a hydrogen atom from these two compounds. At 290 nm, xanthine is known to absorb light. Enzyme activity was determined by monitoring the spectrophotometric increase in uric acid at 290 nm. A 96-well microplate was used for the activity tests. The components of the activity measurement are listed in Table 3.1.

Table 3.1 Method for determining xanthine oxidase enzyme activity

Stock solutions	Preparation	Volume (µL)
0.5 M of Phosphate Buffer	pH = 7.5	50 (µL)
Hypoxanthine	4mg per 10ml (9mL disttel water + 1mL buffer)	60 (µL)
Xanthine		10 (µL)
Disttel water	_	100 (µL)

3.2.2 Studies of enzyme inhibition

To ascertain the impact of copper and palladium transition metal complexes on the xanthine oxidase enzyme's activity, ten times the chemicals were deluted, and the enzyme activity was examined at various doses. We diluted the compounds with a significant inhibitory effect until the enzyme activity was reduced by 50%. Enzyme activity was measured at a range of five concentrations, from below and above the concentration at which activity was halved. Enzyme activity without inhibitors was absorbed as a control and used for analysis. The inhibitors' effect was quantified as a percentage of the total activity. The IC50 values were calculated using these plots.

3.2.3 Molecular docking studies

The inhibitory effects of drugs were uncovered by retrieving their 3D structures in sdf format from the PubMed website (https://pubchem.ncbi.nlm.nih.gov). It was possible to get the human xanthin oxidase enzyme's crystal structure in PDB format from the Protein Data Bank website.

3.2.4 BIOVIA discovery studio docking protocol

The protein has been added to the application. Both the heteroatoms other than the ligand and the water molecules on the protein crystal structure were eliminated a transfer of molecules to the plan (BIOVIA Discovery Studio 2021). For docking, the xanthine oxidase-binding site was identified. An operations hub and docking space for 19 vessels were chosen. As many as 10 docking attempts were attempted by the computer for each ligand. To determine this, we considered the highest MolDock ratings.

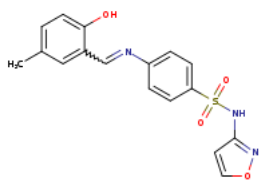
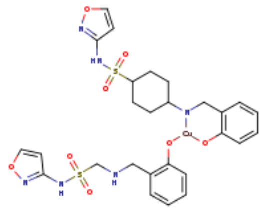
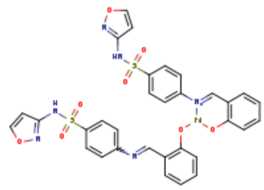
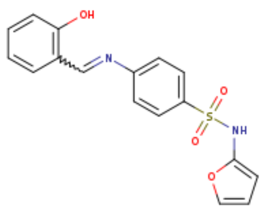
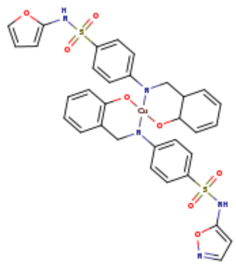
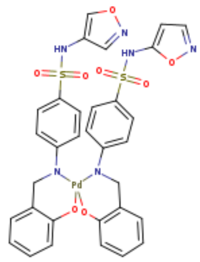
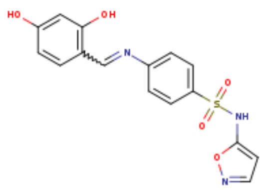
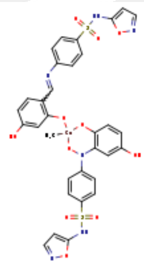
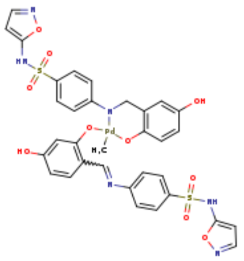
4. RESULTS

We looked at the twelve chemicals' ability to block the xanthine oxidase enzyme. Table 4.1, Table 4.2 and Figure 4.1 displays the in vitro and in silico results.

Table 4.1 Substances' effects on the activity of the xanthine oxidase enzyme

Compounds	IC50 µM	MolDock Score	HBond
S1M-S1	187.8447	-133.708	-6.83065
Pd(S1M-S1)2	0.825372	-214.166	-10.4734
Cu(S1M-S1)2	1.033006	-214.162	-10.4824
S2M-S1	202.0837	-127.922	-3.60766
Pd(S2M-S1)2	33.71338	-211.949	-5.20211
Cu(S2M-S1)2	0.210108	-211.945	-5.20529
S1M-S3	138.0771	-138.483	-7.1174
Pd(S1M-S3)2	15.26756	-201.907	-1.30438
Cu(S1M-S3)2	0.220343	-201.904	-1.30896
S2M-S3	71.01916	-131.024	-5.43181
Pd(S2M-S3)2	0.357661	-204.751	-2.67149
Cu(S2M-S3)2	44.12139	-204.854	-2.93272

Table 4.2 Compounds' structures tested against XO

		
(S1M-S1)	[Cu(S1M-S1)]	[Pd(S1M-S1)]
		
(S2M-S1)	[Cu(S2M-S1)]	[Pd(S2M-S1)]
		
(S2M-S3)	[Cu(S2M-S3)]	[Pd(S2M-S3)]

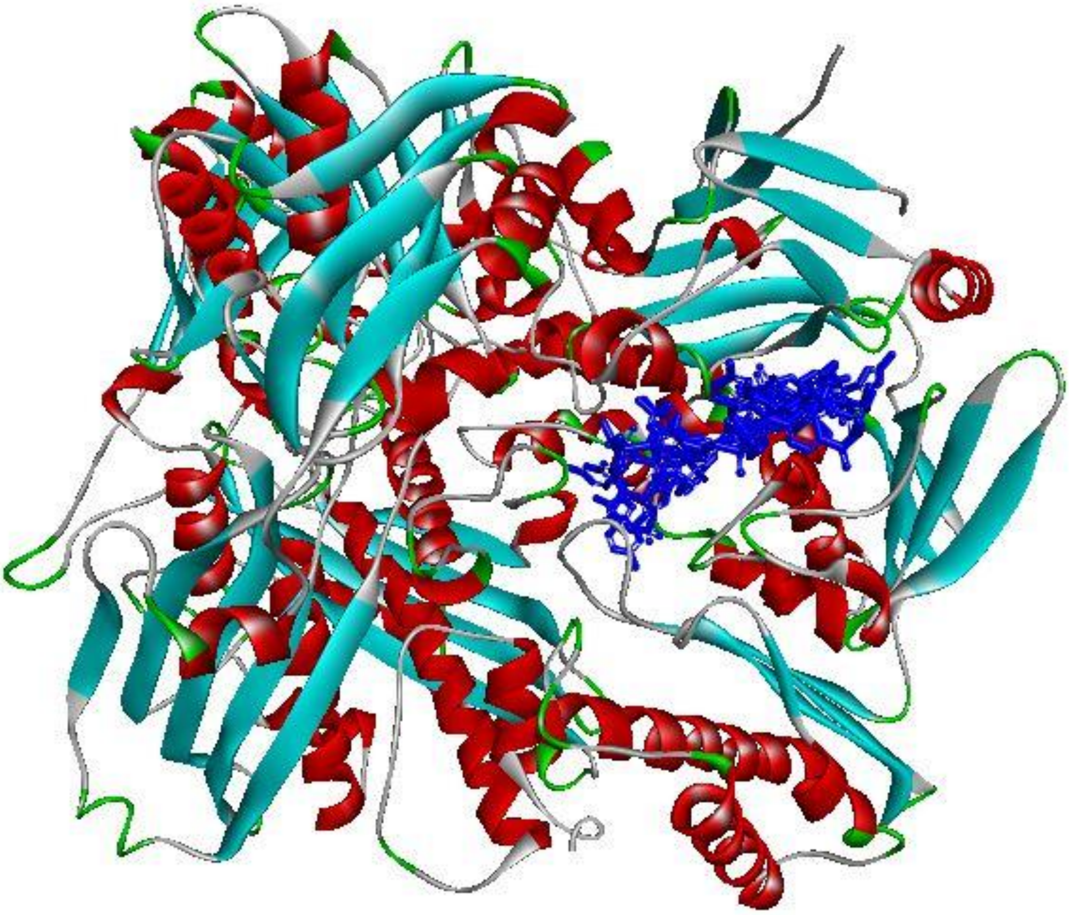


Figure 4.1 Docking area and the XO structure

In the compound (S1M-S1), there were very weak inhibitory effects against the enzyme xanthine oxidase. Figure 4.2 shows a graph of the compound with the value of IC_{50} = 187.8447 and the degree of MolDock (-133.708) this result is expected because the compound does not contain a central metal atom.

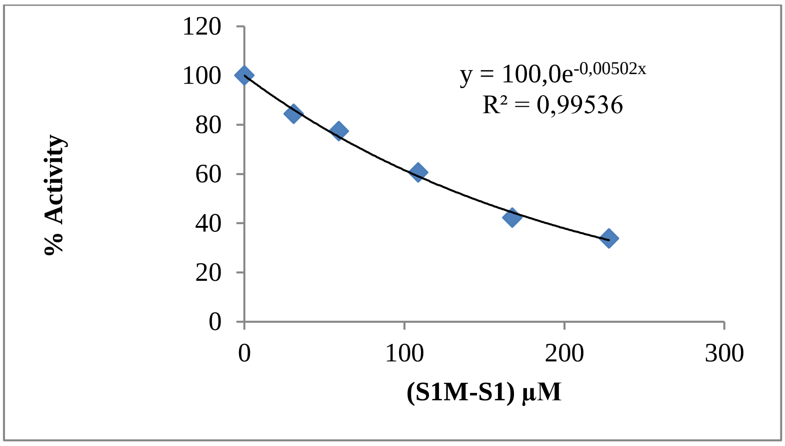


Figure 4.2 Schematic diagram of the effect of compound (S1M-S1) on XO enzyme activity

Where the amino acids (GLY B:260, SER B:347 and LEU B:257) formed Conventional Hydrogen Bond. (THR B:354) formed Carbon Hydrogen Bond. (VAL B:259 and ILE B:353) formed Pi-Alkyl Figure 4.3 .

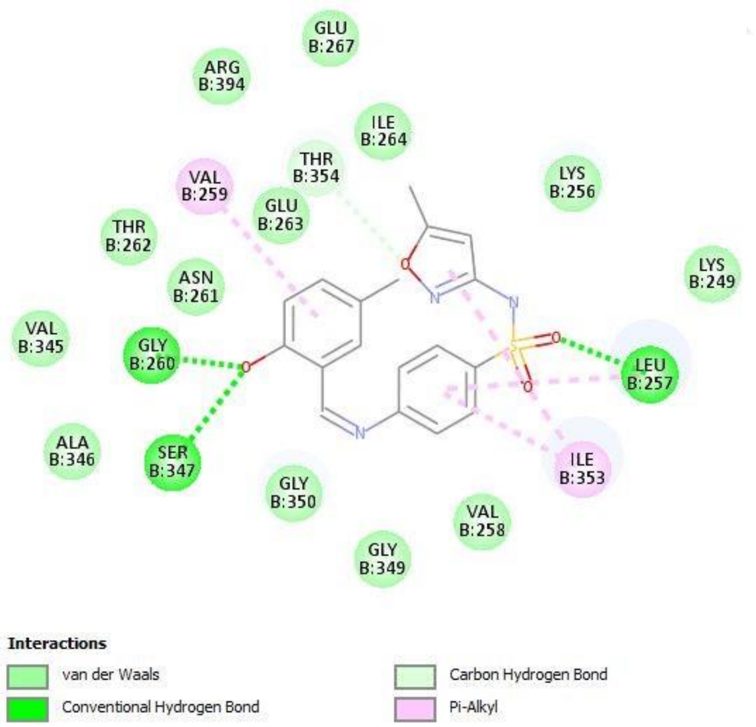


Figure 4.3 The interactions of (S1M-S1) with the active site of XO

The same compound (S1M-S1) after adding a metal atom is palladium to form a metal complex [Pd(S1M-S1)$_2$]. Figure 4.4 shows a graph of the compound with the value of IC_{50} = 0.825372 μM According to the docking results, it was determined as a (-214.166) MolDock Score.

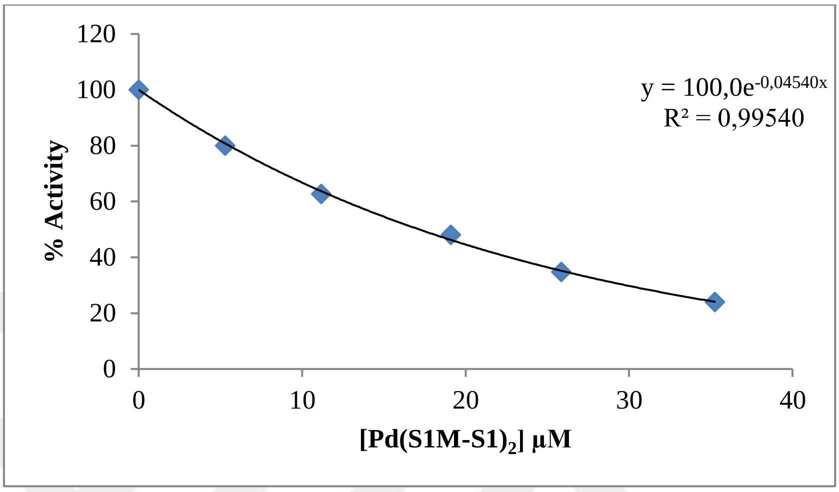


Figure 4.4 Schematic diagram effect of the complex [Pd(S1M-S1)$_2$] on XO enzyme activity

Schematic diagram of the effect of [Pd(S1M-S1)2] on xanthine oxidase activity.When examining the three-dimensional interaction map with the enzyme xanthine oxidase Figure 4.5 the amino acids (GLU B:263,THR B:262, GLY A:46 and TRP B:336) formed Unfavorable Bump (ASN B:261, VAL B:259, LYS B:433 and ALA B:338) formed Conventiona; Hydrogene Bond (GLY B:350 and PHE B:337) Carbon Hydrogen Bond (ILE B:353, ALA B:346, LEU B:305, VAL B:342, and LEU A:74) Alkyl and Pi-alkyl and several van der waals bond.

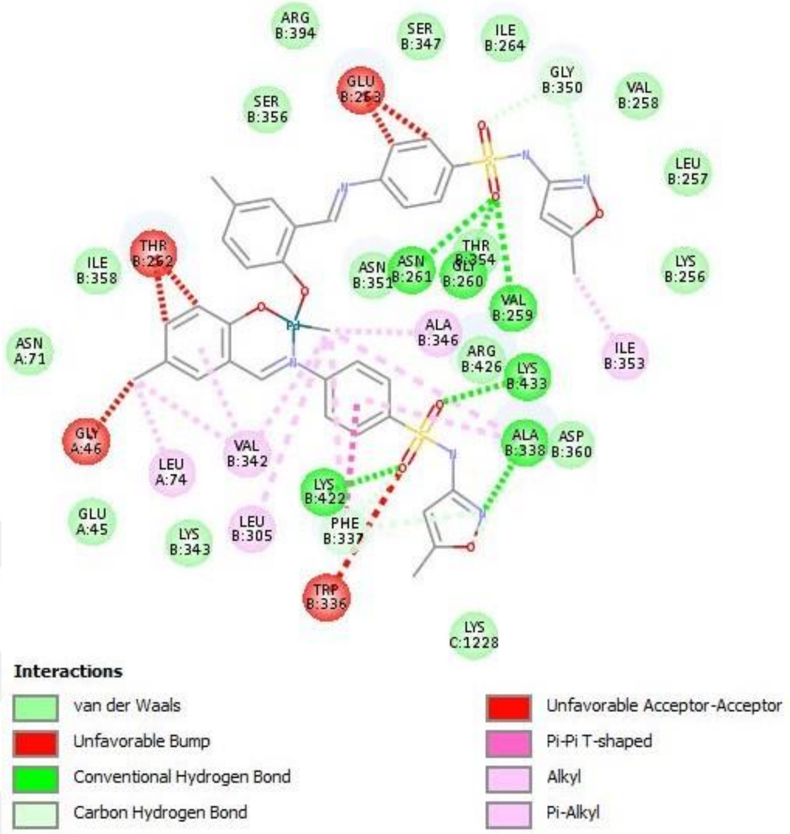


Figure 4.5 The interactions of [Pd(S1M-S1)$_2$] with the active site of XO

The same compound (S1M-S1) after adding a metal atom is copper to form a metal compound [Cu(S1M-S1)$_2$]. Figure 4.6 shows a graph of the compound with a value of IC50 = 1.033006 µM according to the docking results, identified as Moldoc score (-214.162).

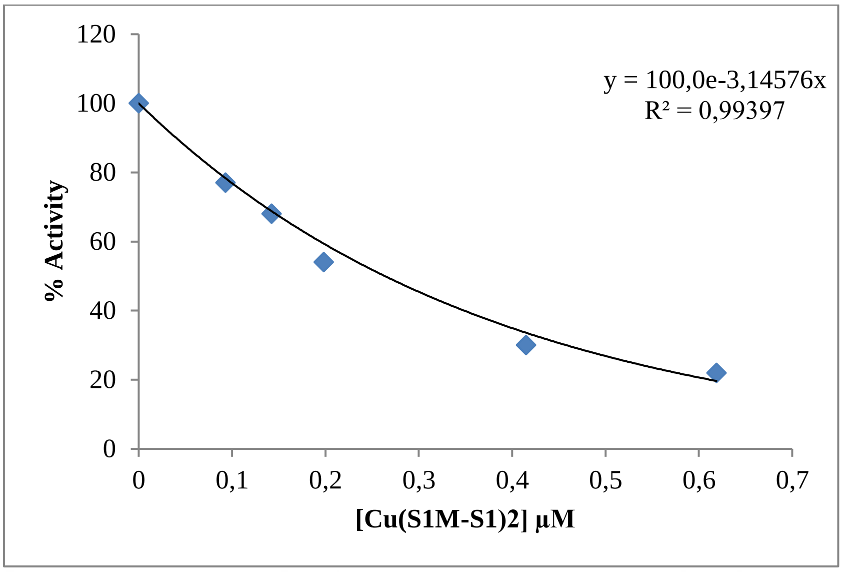


Figure 4.6 Schematic diagram effect of the complex [Cu(S1M-S1)$_2$] on XO enzyme activity

When examining the three-dimensional interaction map with the enzyme xanthine oxidase figure 4.7 the amino acids (GLU B:263,THR B:262, GLY A:46 and TRP B:336) formed Unfavorable Bump (ASN B:261, VAL B:259, LYS B:433 and ALA B:338) formed Conventiona; Hydrogene Bond (GLY B:350 and PHE B:337) Carbon Hydrogen Bond (ILE B:353, ALA B:346, LEU B:305, VAL B:342, and LEU A:74) Alkyl and Pi-alkyl and several van der waals bond

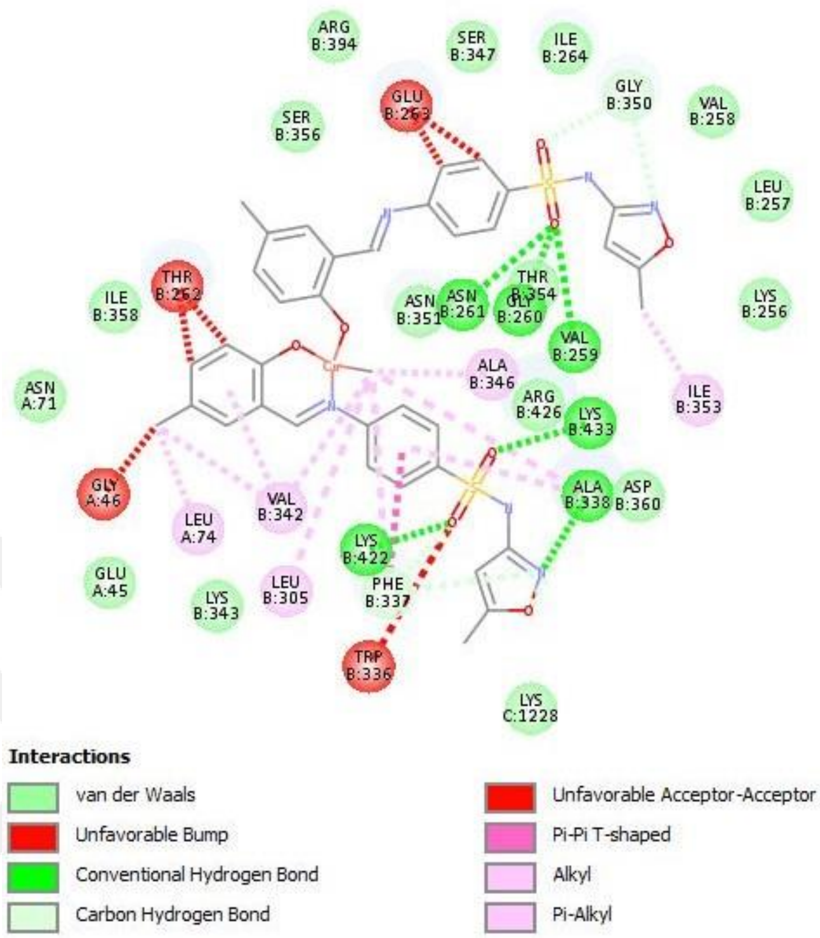


Figure 4.7 The interactions of [Cu(S1M-S1)$_2$] with the active site of XO

In the compound (S2M-S1), there were very weak inhibitory effects against the enzyme xanthine oxidase. Figure 4.8 shows a graph of the compound with the value of IC50 = 202.0837 μM and the degree of MolDock Score (-127.922) this result is expected because the compound does not contain a central metal atom.

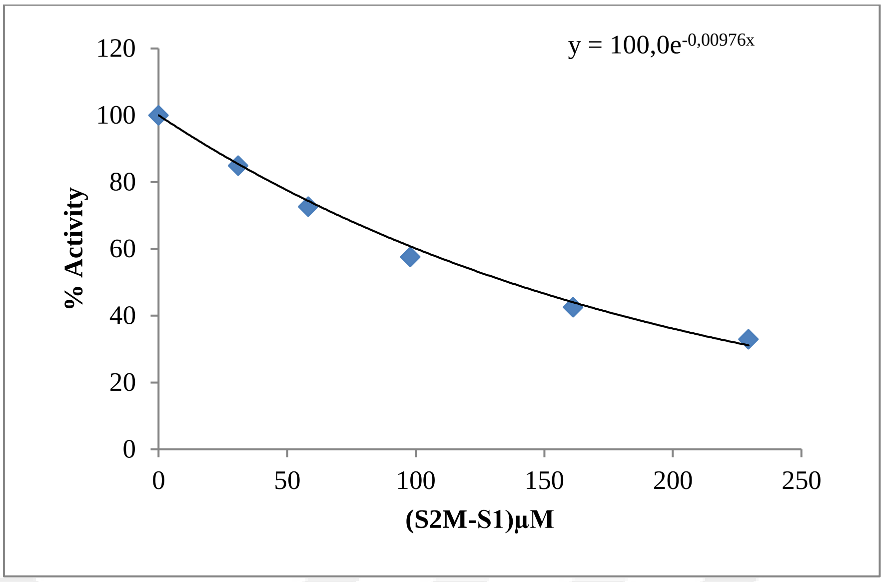


Figure 4.8 Schematic diagram of the effect of compound (S2M-S1) on XO enzyme activity

3D reaction map examination with xanthine oxidase enzyme showed Figure 4.9 amino acids (ASN B:261, VAL B:259, and LYS B:256) formed Conventional Hydrogen Bond (GLY B:350) formed Carbone Hydrogen Bond (LEU B:257 and ILE B:264) formed Pi-alkyl and many Bond van der Waals.

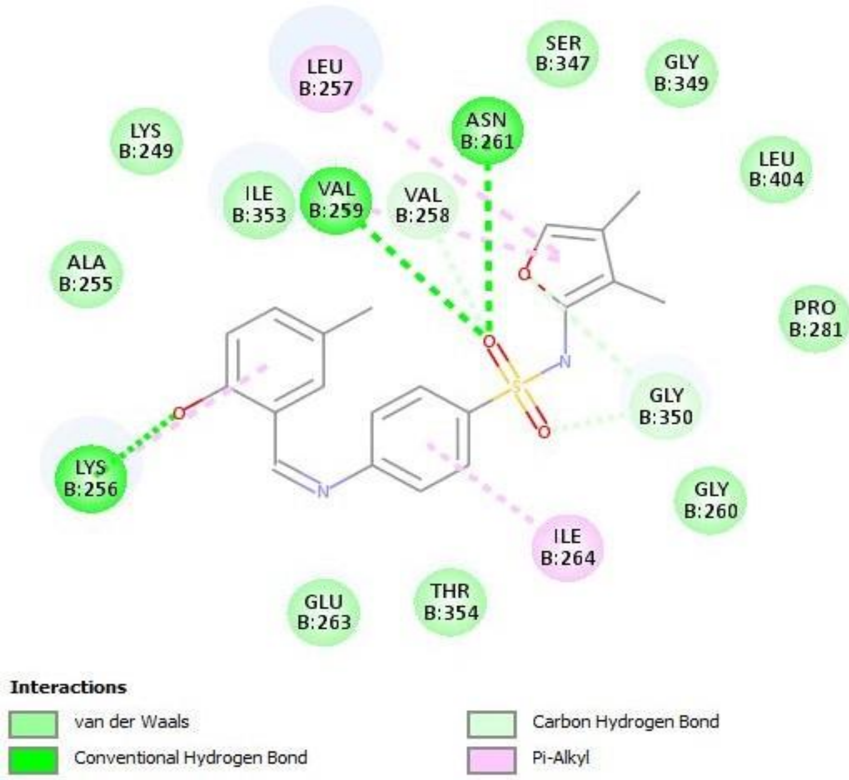


Figure 4.9 The interactions of (S2M-S1) with the active site of XO

The same compound (S2M-S1) after adding a metal atom is palladium to form a metal complex [Pd(S2M-S1)$_2$]. Figure 4.10 shows a graph of the compound with the value of IC_{50} = 33.71338 µM According to the docking results, it was determined as a (-211.949) MolDock score.

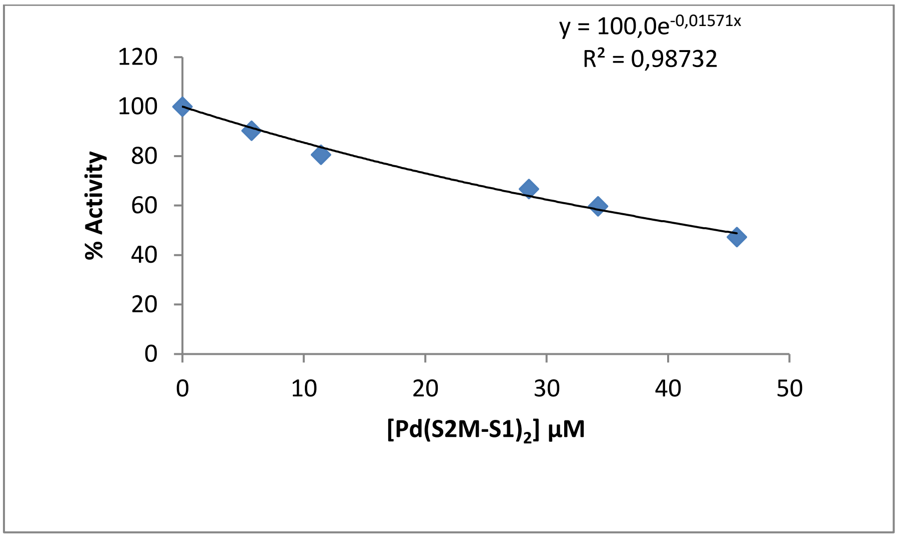


Figure 4.10 Schematic diagram effect of the complex [Pd(S2M-S1)$_2$] on XO enzyme activity

A 3D reaction map examination with xanthine oxidase showed in Figure 4.11 amino acids (GLU A: 45, LEU A: 74, and TRP B: 336) formed an unfavorable bump (THR B:262, ALA B:338 and SER C:1225) formed the convention Hydrogen Bond (GLU B:263 and LYS C:1228) formed Pi-cation and Pi-Anion (THR B:262) Pi-lone Pair (LYS B:422, LYS B:433, VAL B:342 LEU A:147) Alkyl and Pi-alkyl and many Bond van der Waals.

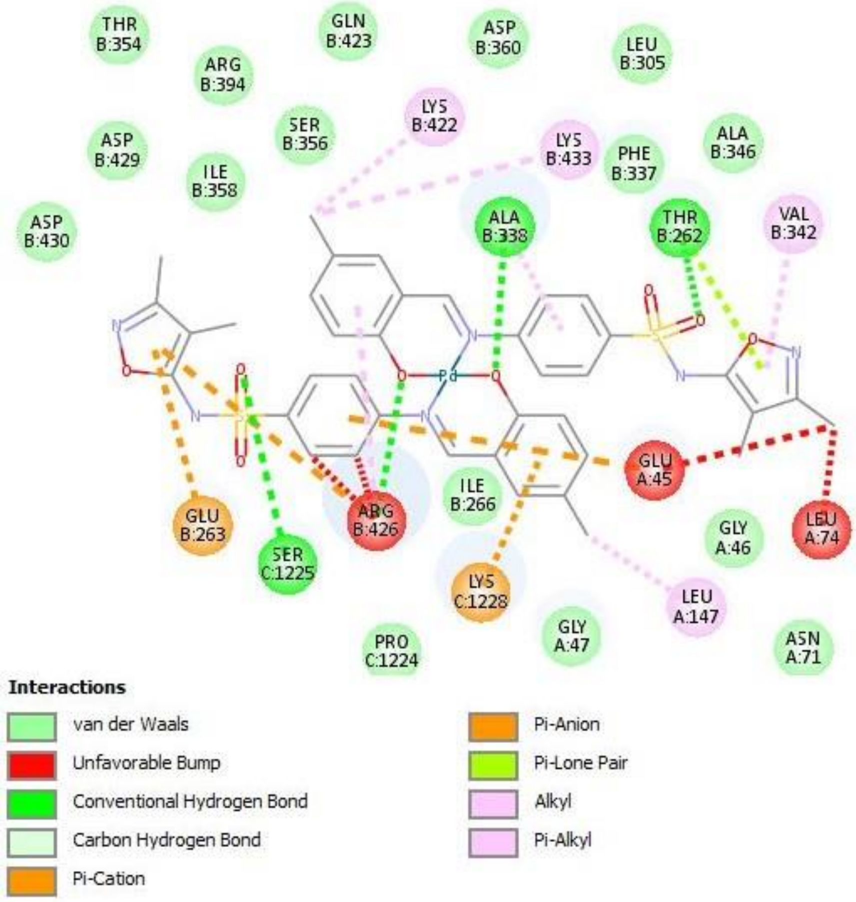


Figure 4.11 The interactions of [Pd(S2M-S1)$_2$] with the active site of XO

The same compound (S2M-S1) after adding a metal atom is copper to form a metal complex [Cu(S2M-S1)$_2$]. Figure 4.12 shows a graph of the compound with the value of IC50 = 0.210108 μM According to the docking results, it was determined as a (-211.945) MolDock Score.

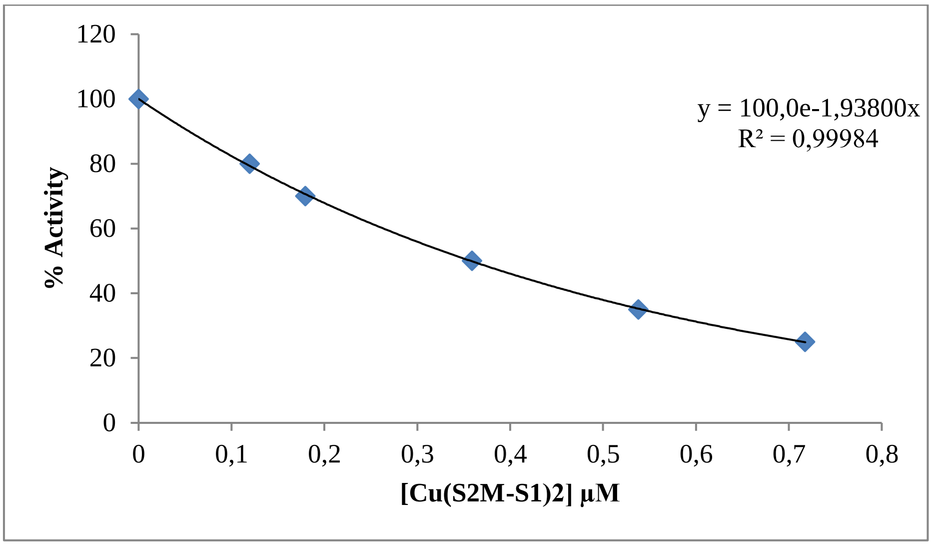


Figure 4.12 Schematic diagram effect of the complex [Cu(S2M-S1)$_2$] on XO enzyme activity

A 3D reaction map examination with xanthine oxidase showed in Figure 4.13 amino acids (GLU A: 45, LEU A: 74, and TRP B: 336) formed an unfavorable bump (THR B:262, ALA B:338 and SER C:1225) formed the convention Hydrogen Bond (GLU B:263 and LYS C:1228) formed Pi-cation and Pi-Anion (THR B:262) Pi-lone Pair (LYS B:422, LYS B:433, VAL B:342 LEU A:147) Alkyl and Pi-alkyl and many Bond van der Waals

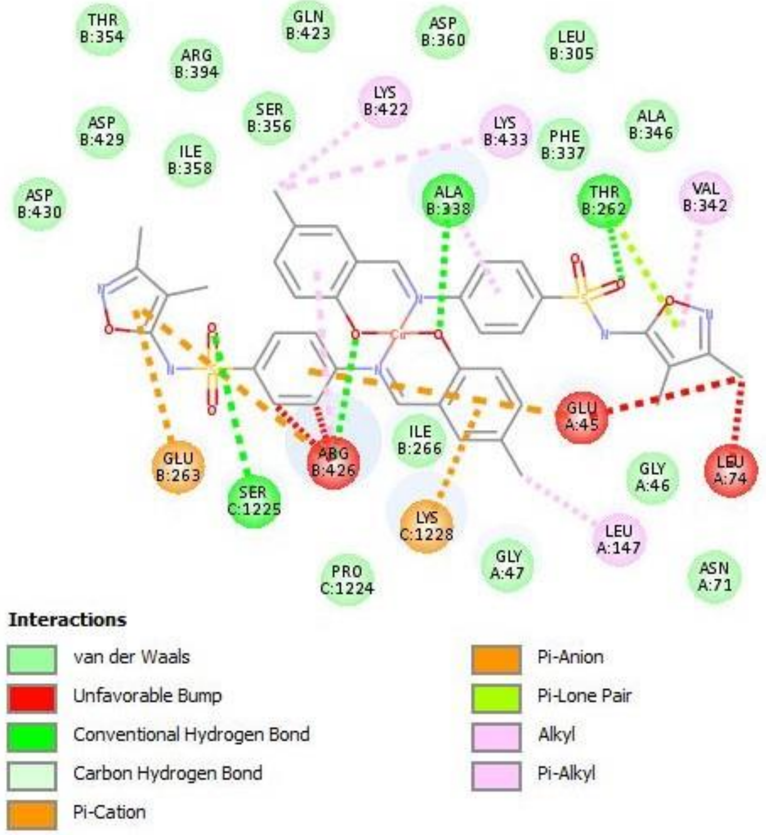


Figure 4.13 The interactions of [Cu(S2M-S1)$_2$] with the active site of XO

The compound (SIM-S3) showed very weak inhibitory effects against the xanthine oxidase enzyme Figure 4.14 displays a [S1M-S3] graph with a IC_{50}= 138.0771 μM value. The docking findings showed that it had a MolDock Score of (-138.483), and this result is expected that it does not contain any metal atom.

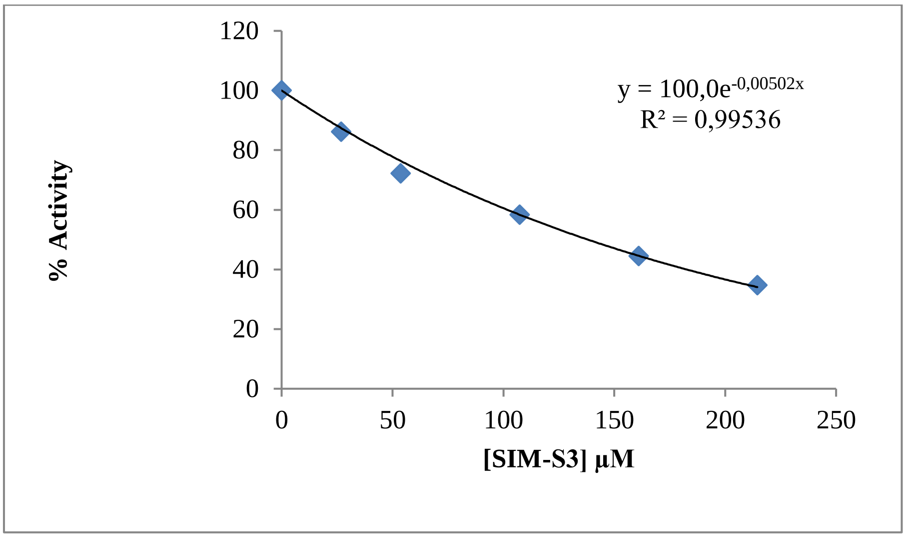


Figure 4.14 Schematic diagram of the effect of compound (S1M-S3) on XO enzyme activity

When the 3D reaction map with the enzyme is examined, amino acids GLY B:350, SER B:347, GLY B:260, GLU B:263, ILU B:264, and LEU B:404 performed Conventional Hydrogen Bond amino acid ILE B;353 and VAL B:259 performed Unfavorable Bump bond, ALA B:302, ALA B:301, PRO B:281, and LEU B:257 performed alkyl and Pi-alkyl bond and many Van der Waals bond bonding interactions with (S1M-S3). In Figure 4.15

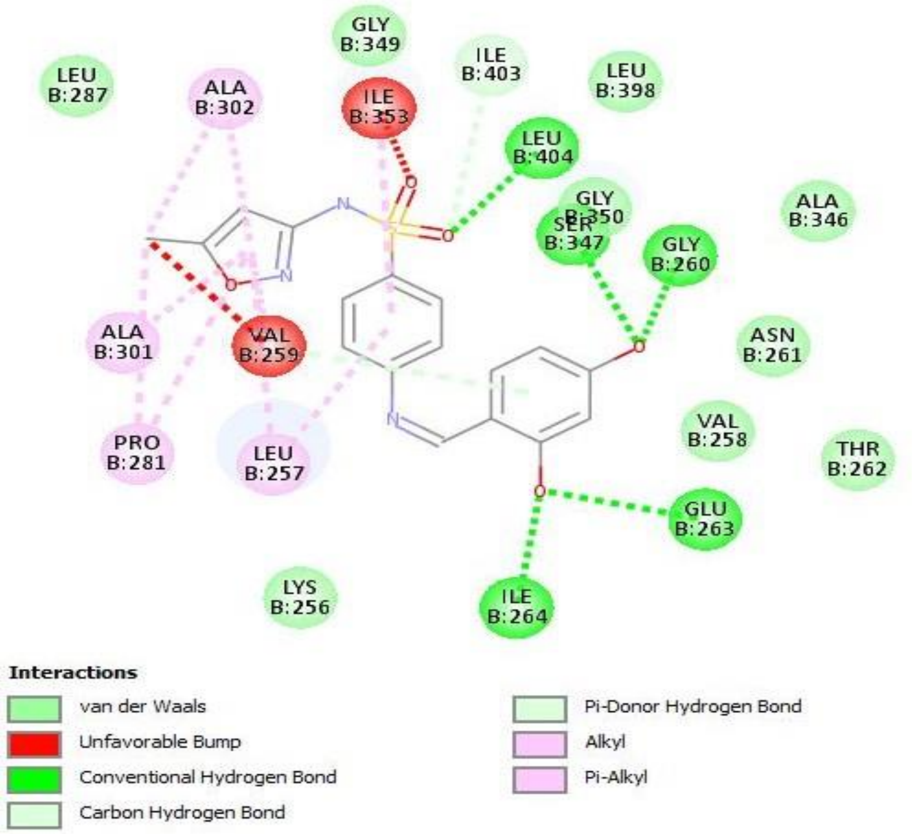


Figure 4.15 The interactions of (S1M-S3) with the active site of XO

With an IC_{50} value of 15.26 µM, the chemical Pd(S1M-S3)$_2$ showed inhibitory effects on the xanthine oxidase enzyme. Activity % [Pt(S1M-S3)$_2$] Figure 4.16 showed a 3D interaction maps. It had a (-201.907) MolDock score, as assessed by the docking results.

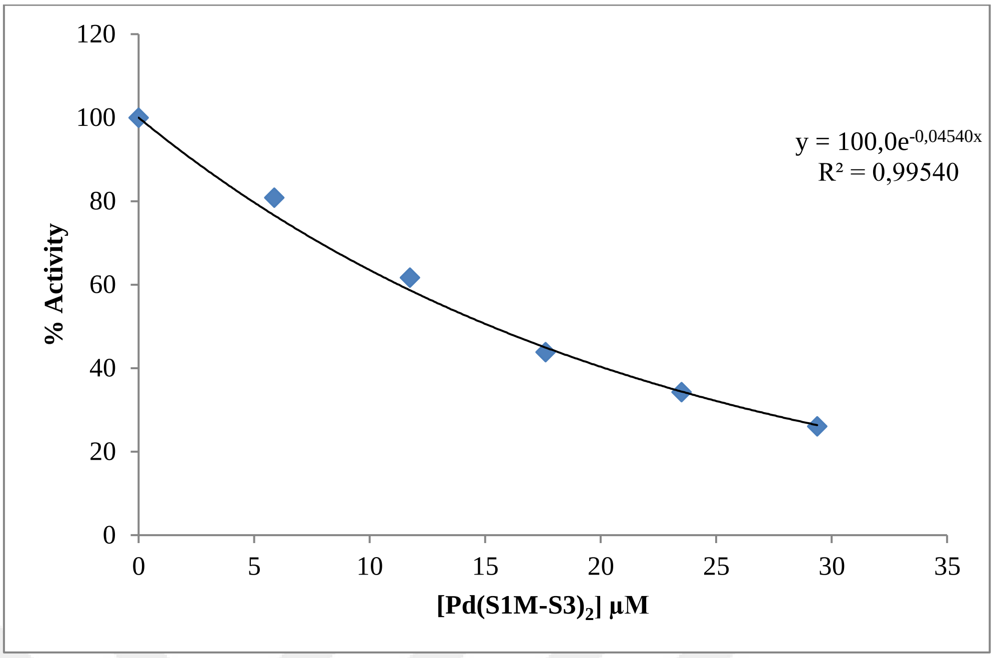


Figure 4.16 Schematic diagram effect of the complex [Pd(S1M-S3)$_2$] on XO enzyme activity

When the 3D interaction map with the enzyme is examined, the amino acids GLY B: 350 and SER B: 347 performed the conventional hydrogen bond amino acids; 353 and LEU B: 245 unfavorable collision associations were made, LYS B: 271, AGR B: 394, ILE B: 264, LEU B: 398, PRO B: 281, ILE B: 403, LEU B: 287, and LEU B:257 performed alkyl and Pi-alkyl bonds GLY B:349, LYS B:256 of carbone hydrogen bond and several Van der Waals bond interactions with Pd(S1M-S3). In Figure 4.17

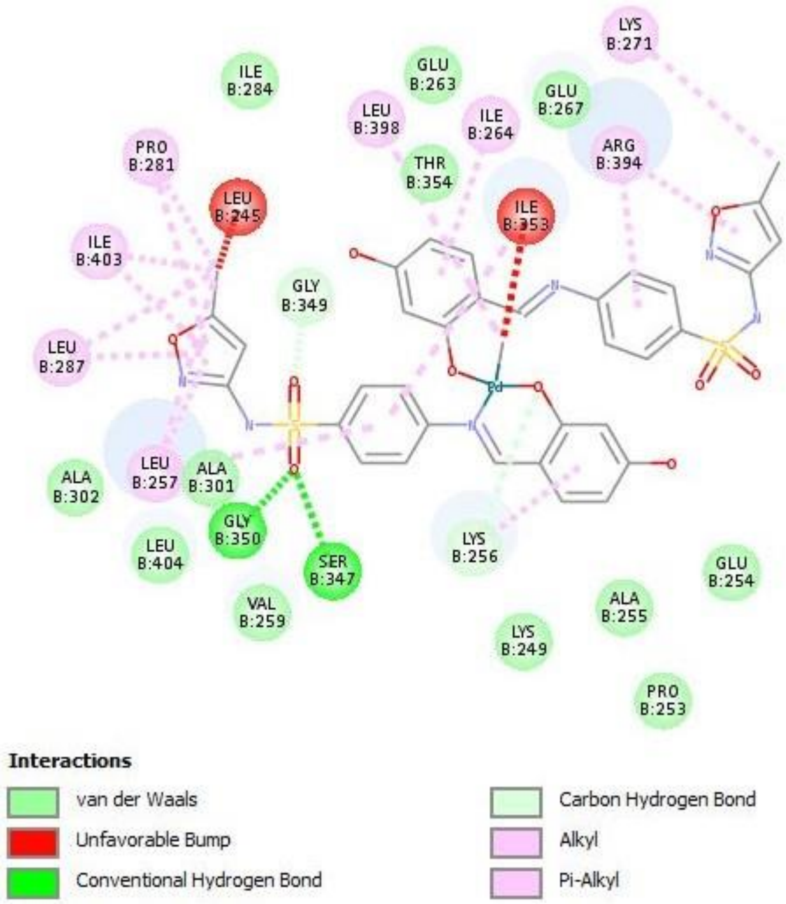


Figure 4.17 The interactions of [Pd(S1M-S3)$_2$] with the active site of XO

With an IC_{50} value of 0.220343 μM, the chemical Cu(S1M-S3)$_2$ showed inhibitory effects on the xanthine oxidase enzyme. Activity % [Cu(S1M-S3)$_2$] Figure 4.18 showed a 3D interaction maps. It had a (-201.904) MolDock Score, as assessed by the docking results.

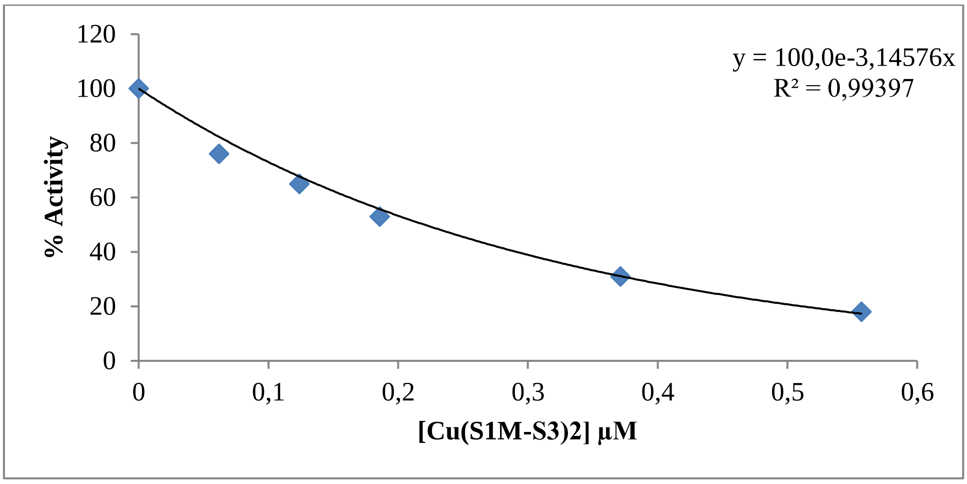


Figure 4.18 Schematic diagram effect of the complex [Cu(S1M-S3)$_2$] on XO enzyme activity

When the 3D interaction map with the enzyme is examined, the amino acids GLY B: 350 and SER B: 347 performed the conventional hydrogen bond amino acids, ILE B:353 and LEU B: 245 unfavorable Bump collision associations were made, LYS B: 271, AGR B: 394, ILE B: 264, LEU B: 398, PRO B: 281, ILE B: 403, LEU B: 287, and LEU B:257 performed alkyl and Pi-alkyl bonds GLY B:349, LYS B:256 of carbon-hydrogen bond and several Van der Waals bond interactions with Cu(S1M-S3). In Figure 4.19

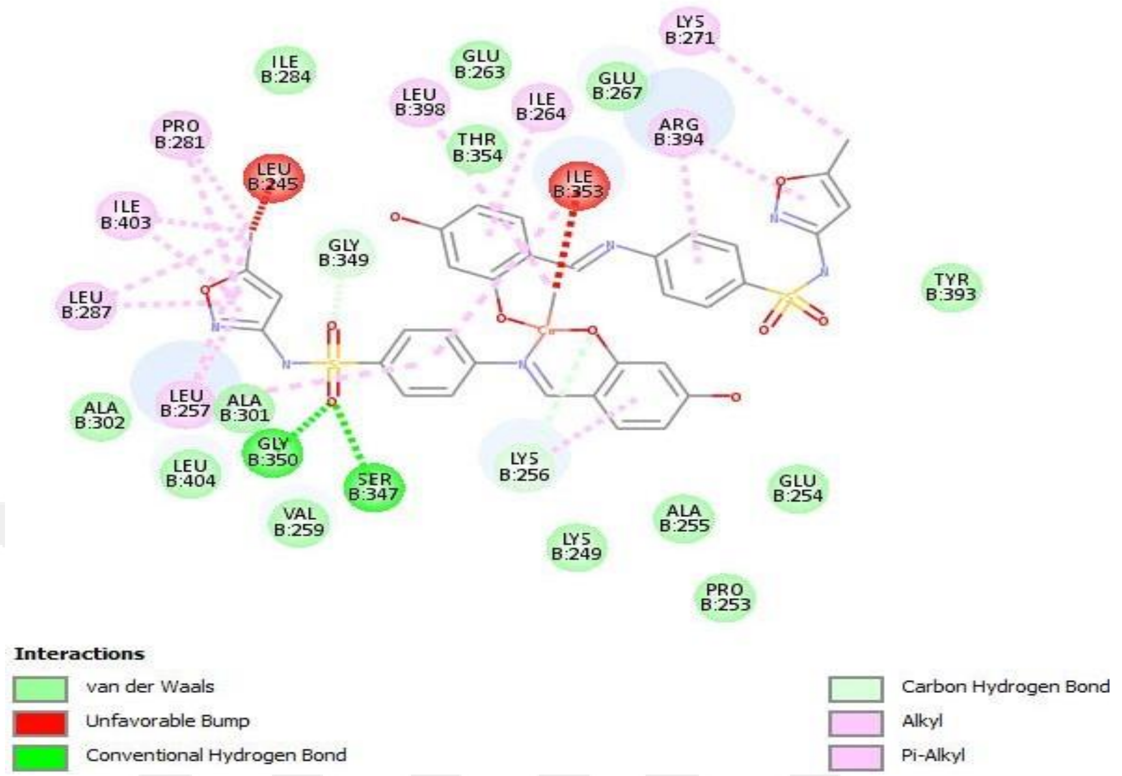


Figure 4.19 The interactions of [Cu(S1M-S3)$_2$] with the active site of XO

The compound (S2M-S3) showed very weak inhibitory effects against the xanthine oxidase enzyme Figure 4.20 displays a [S2M-S3] graph with a IC_{50}= 71.01916 µM value. Figure 4.19 displays 3D interaction maps. The docking findings showed that it had a MolDock score of a (-131.024) and this result is expected that it does not contain any metal atom.

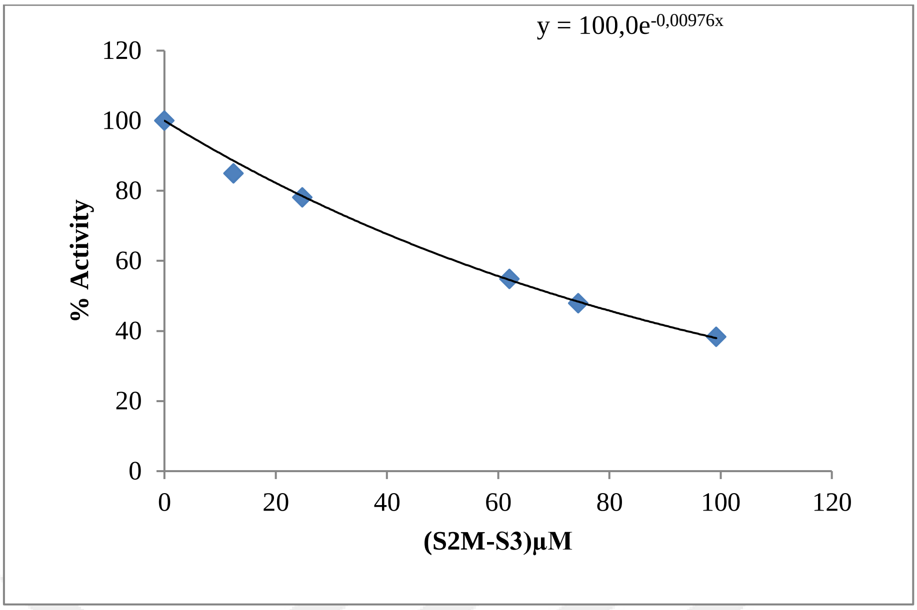


Figure 4.20 Schematic diagram of the effect of compound (S2M-S3) on XO enzyme activity

When the 3D reaction map with the enzyme is examined, amino acids (LEU B:404, GLY B:350, GLY B:349, ALA B:301. SER B:347, and LEU B:257) performed Conventional Hydrogen Bond amino acid LYS B:256 a performed Pi-Cation bond, Pi-Alkyl with tow amino acid LEU B:257 and ALA B:301, carbon hydrogen bond with THR B:354, ALA B:302 bonding interactions with (S2M-S3). In Figure 4.21

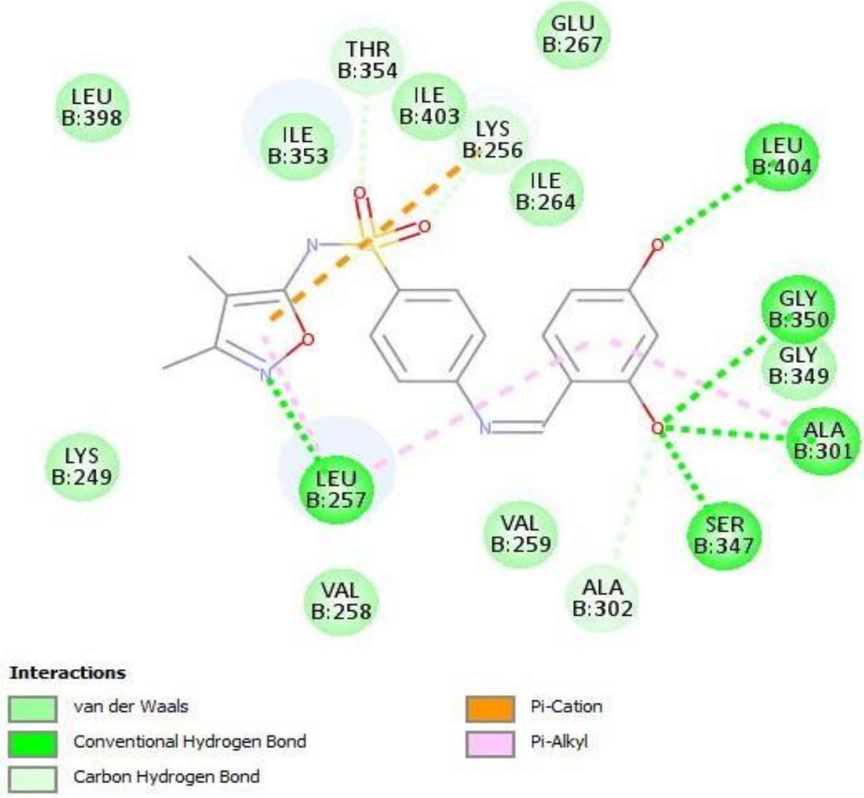


Figure 4.21 The interactions of (S2M-S3) with the active site of XO

With an IC_{50} value of 0.3576 µM, the chemical Pd(S2M-S3)$_2$ showed inhibitory effects on the xanthine oxidase enzyme. Activity % [Pt(S2M-S3)$_2$] Figure 4.22 showed a 3D interaction maps. It had a (-204.751) MolDock score, as assessed by the docking results

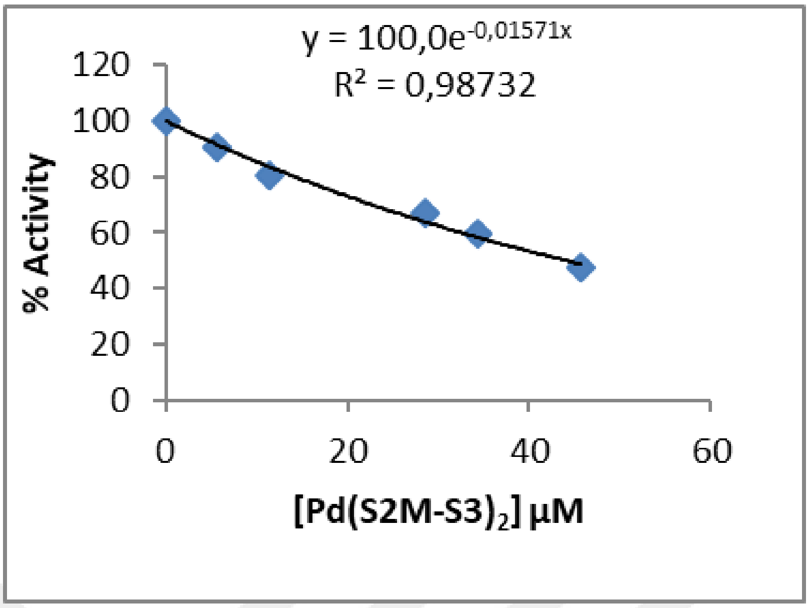


Figure 4.22 Schematic diagram effect of the complex [Pd(S2M-S3)$_2$] on XO enzyme activity

When the 3D reaction map with the enzyme is examined, amino acids (THR B:396) performed Conventional Hydrogen Bond (ARG B:394 and ASP B:429) a performed Pi-Cation and Pi-Anion bond, Pi-Alkyl and Alky with amino acids ILU B:266, LYS B:271, and ARG B:394 Unfavorable Bump with (LYS B:271 Pi-Sigma PRO B:400) Figure 4.23.

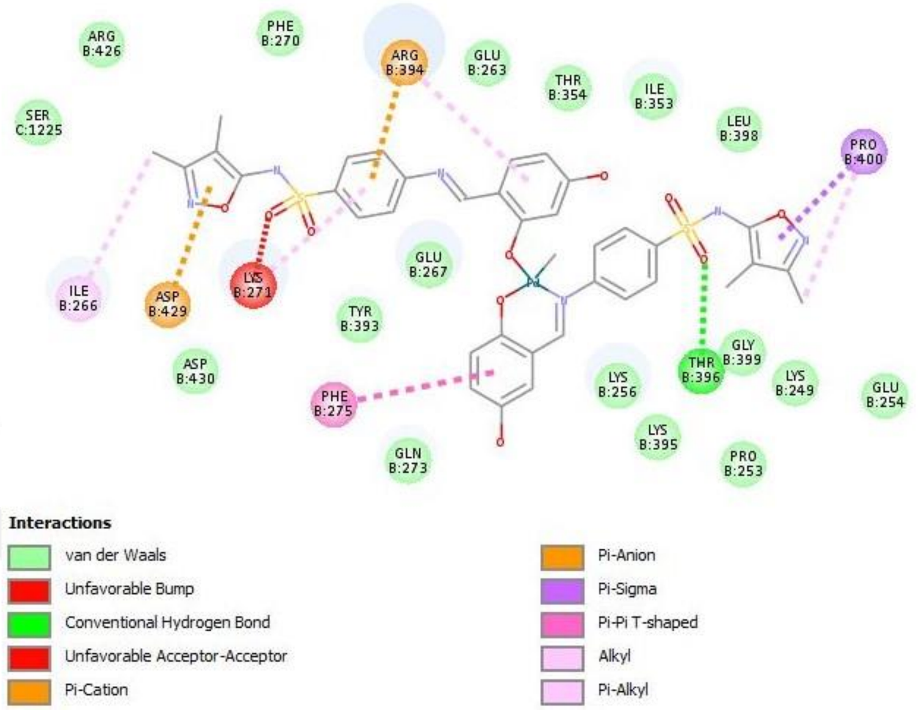


Figure 4.23 The interactions of [Pd(S2M-S3)$_2$] with the active site of XO

With an IC50 value of 44.12139 μM, the chemical Cu(S2M-S3)$_2$ showed inhibitory effects on the xanthine oxidase enzyme. Activity % [Cu(S2M-S3)$_2$] Figure 4.24 showed a 3D interaction maps. It had a (-204.854) MolDock score, as assessed by the docking results.

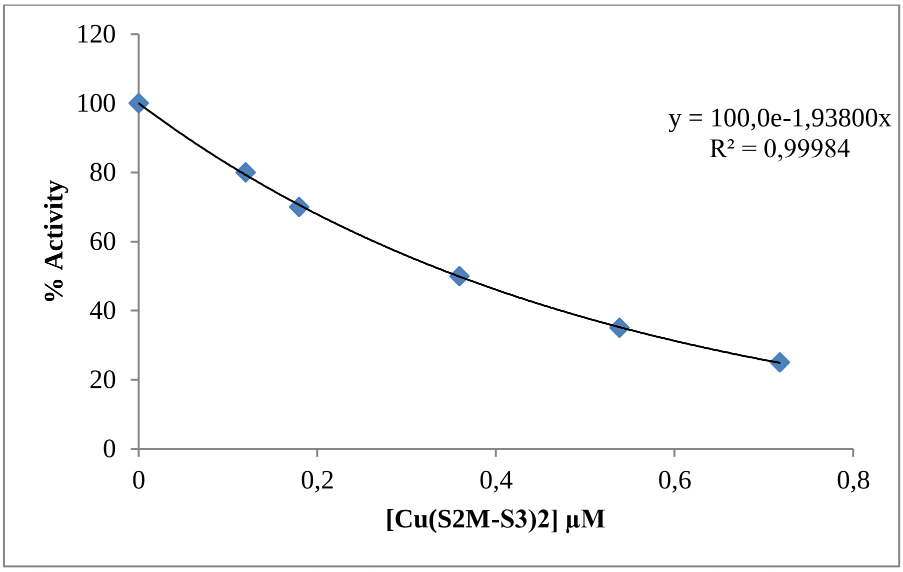


Figure 4.24 Schematic diagram effect of the complex [Cu(S2M-S3)$_2$] on XO enzyme activity

When the 3D reaction map with the enzyme is examined, amino acids LYS B:271 performed an Unfavorable Bump bond THR B:396 Conventional hydrogen bond ARG B:394 and ASP B:429 Pi-Catioan and Pi-Anion PRO B:400 performed Pi-Sigma PHE B:275 made Pi-Pi T-shaped ILE B:266 performed Alkyl and Pi-Alkyl bond and several of van der Waals bond in Figure 4.25.

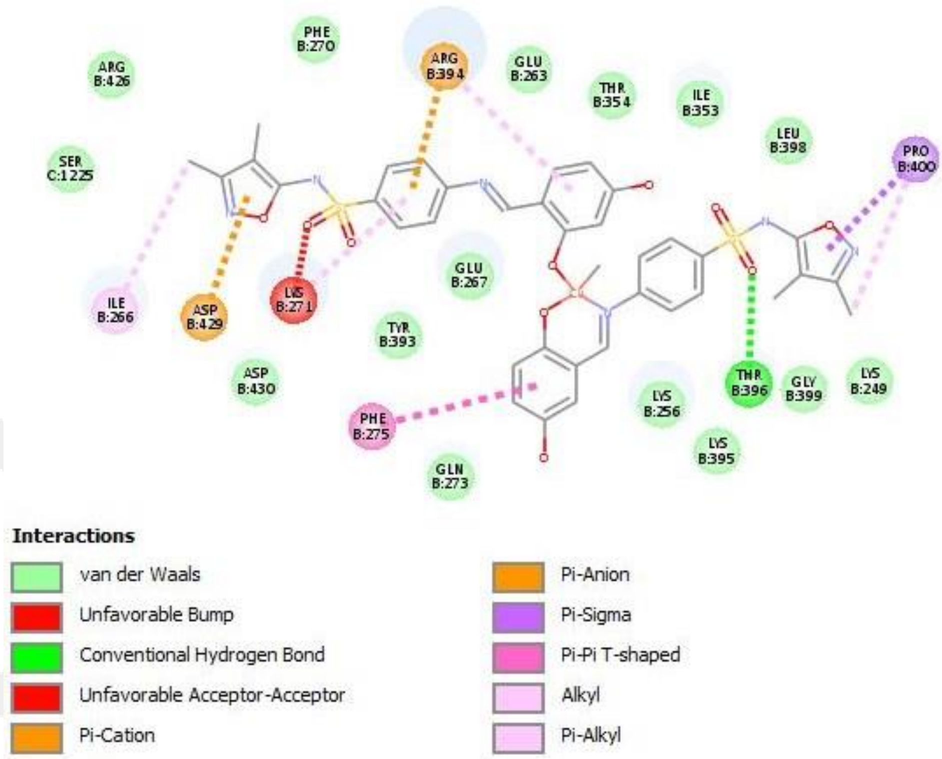


Figure 4.25 The interactions of [Cu(S2M-S3)$_2$] with the active site of XO

.

5. CONCLUSIONS AND DISCUSSION

There is agreement that xanthine oxidase levels are significantly increased in various pathological conditions such as hepatitis, heart disease, cancer, old age, atherosclerosis and Alzheimer's dementia besides the main disease, which is gout due to an increase in uric acid in the blood, which leads to the formation of small crystals in the joints and kidneys. . Therefore, inhibition of this enzyme may be beneficial for exposure to a large number of metabolites from the environment, such as exposure to UV, X-rays and gamma rays. In particular, it has a considerable effect on oxidative stress, which arises from a discrepancy between antioxidant systems and the formation of oxidants such reactive oxygen species (ROS) (reactive oxygen species).

It was found that the compound (S1M-S1) inhibition of concentration equal to 187.844 μM in Figure 4.3 and its complexities [Pd(S1M-S1)$_2$] inhibition of concentration equal to 0.8253 μM figure 4.5 and its second complexities [Cu(S1M-S1)$_2$] inhibition of concentration equal to 1.033μM in figure 4.7 And the compound (S2M-S3) inhibition of concentration equal to 71.019 μM in figure 4.21 and its complexities [Pd(S2M-S3)$_2$] inhibition of concentration equal to 0.3576 μM in figure 4.23 and its second complexities [Cu(S2M-S3)$_2$] inhibition of concentration equal to 44.1213 μM in figure 4.25 . All of the above compounds are palladium complexes that have higher inhibition than copper complexes, noting that some results are acceptable for both complexes.

And the compound (S2M-S1) inhibition of concentration equal to 202.083 μM in Figure 4.9 and its complexities [Pd(S2M-S1)$_2$] inhibition of concentration equal to 33.7133 μM figure 4.11 and its second complexities [Cu(S2M-S1)$_2$] inhibition of concentration equal to 0.2101μM in figure 4.13 And the compound (S1M-S3) inhibition of concentration equal to 138.077 μM in figure 4.15 and its complexities [Pd(S2M-S3)$_2$] inhibition of concentration equal to 15.2675 μM in figure 4.17 and its second complexities [Cu(S2M-S3)$_2$] inhibition of concentration equal to 0220343 μM in figure 4.19 As for these compounds, the highest inhibition was for copper complexes.

This difference in results is due to the fact that the bonding of the central atoms in the palladium and copper compounds to the amino acid in the enzyme differs, for example, if palladium bonds with (-NH_2), then copper bonds with oxygen for carboxylic of the amino acid (-COOH) or copper bonds with (-NH_2), then palladium bonds with oxygen for carboxylic of the amino acid (-COOH) , It also depends on the nature and composition of the compounds involved in the enzymatic reaction.

The nitrogen atom in the amino acid does not participate in the formation of the complex, so we can conclude that the copper and palladium ions are linked to the amino acid through the carboxylate ion through the oxygen atom.

All compounds that contain a metal atom (metal complex) behave as an enzyme inhibitor, although the degree of inhibition varies from one compound to another, and this depends on the form of the compound, as well as the number and method of hydrogen bonding between the metal complex (palladium or copper) and the amino acid of the enzyme. These complexes are also considered scavengers of free radicals because, as mentioned in the literature, oxygen radicals O_2^- are a by-product of uric acid, meaning that there is a direct relationship between increased production of uric acid and free radicals or superoxide. Through these complexes, stable free radicals are controlled through electron transfer reactions, and thus as a final outcome Obtaining a drug that has the potential to inhibit the enzyme xanthine oxidase, which in turn reduces the production of uric acid and also the elimination of reactive oxygen species.

In general, the result of the complex [Cu(S2M-S1)$_2$]was better inhibition To determine whether or if this technology can be employed in the medical field, more research is required.

REFERENCES

Buldurun, K., Turan, N., Bursal, E., Mantarcı, A., Turkan, F., Taslimi, P. and Gülçin, İ. 2020. Synthesis, spectroscopic properties, crystal structures, antioxidant activities and enzyme inhibition determination of Co(II) and Fe(II) complexes of Schiff base. Research on Chemical Intermediates, 46(1): 283–297.

Burdon, R. H. 1995. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radical Biology and Medicine, 18(4): 775–794.

Burini, O. 2012. High Plasma Uric Acid Concentration: Causes and Consequences. Diabetology and Metabolic Syndrome, 4(12): 1–7.

Cadenas, E. 1989. Biochemistry of oxygen toxicity. Annual Review of Biochemistry, 58(1): 79–110.

Cadenas, E. and Davies, K. J. A. 2000. Mitochondrial free radical generation, oxidative stress, and aging. Free Radical Biology and Medicine, 29(3–4): 222–230.

Chaudhary, K., Malhotra, K., Sowers, J. and Aroor, A. 2013. Uric acid-key ingredient in the recipe for cardiorenal metabolic syndrome. CardioRenal Medicine, 3(3): 208–220.

Cheng, X. Y., Huang, W. J., Hu, S. C., Zhang, H. L., Wang, H., Zhang, J. X., Lin, H. H., Chen, Y. Z., Zou, Q. and Ji, Z. L. 2012. A global characterization and identification of multifunctional enzymes. PLoS ONE, 7(6): 1–8.

Clémençon, B., Lüscher, B. P., Fine, M., Baumann, M. U., Surbek, D. V., Bonny, O. and Hediger, M. A. 2014. Expression, purification, and structural insights for the human uric acid transporter, GLUT9, The Xenopus laevis Oocytes System. PLoS ONE, 2: 9-10.

Hadizadeh, M., Keyhani, E., Keyhani, J. and Khodadadi, C. 2009. Functional and structural alterations induced by copper in xanthine oxidase. Acta Biochimica Biophysica Sinica, 41(7): 603–617.

Harrison, R. 2002. Structure and function of xanthine oxidoreductase: Where are we now? Free Radical Biology and Medicine, 33(6): 774–797.

Hofman, P., Venissac, N., Hofman, V. and Mouroux, J. 2007. Intérêt d’une collection tissulaire ciblée dans le programme de recherche des tumorothèques: L’exemple des carcinomes pulmonaires. Annales de Pathologie, 27(1): 167–172.

Ikram, M., Rehman, S., Khan, A., Baker, R. J., Hofer, T. S., Subhan, F., Qayum, M., Faridoon, A. and Schulzke, C. 2015. Synthesis, characterization, antioxidant and selective xanthine oxidase inhibitory studies of transition metal complexes of novel amino acid bearing Schiff base ligand. Inorganica Chimica Acta, 428: 117–126.

Jin, M., Yang, F., Yang, I., Yin, Y., Luo, J. J., Wang, H. and Yang, X. F. 2012. Uric acid, hyperuricemia and vascular diseases. Frontiers in Bioscience, 17(2): 656–669.

Johar, S., MacCarthy, P. A. and Shah, A. M. 2006. Oxidative stress and cardiovascular disease. Oxidative Stress, Disease and Cancer, 23(2): 519–536.

Kostić, D. A., Dimitrijević, D. S., Stojanović, G. S., Palić, I. R., Dordević, A. S. and Ickovski, J. D. 2015. Xanthine oxidase: Isolation, assays of activity, and inhibition. Journal of Chemistry, 2: 1-5.

Kostova, I. 2005. Lanthanides as anticancer agents. Current Medicinal Chemistry-Anti-Cancer Agents, 5(6): 591–602.

Laguerre, M., Lecomte, J. and Villeneuve, P. 2007. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in Lipid Research, 46(5): 244–282.

Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C. and Mollace, V. 2016. Regulation of uric acid metabolism and excretion. International Journal of Cardiology, 213: 8–14.

Matsuo, H., Nakayama, A., Sakiyama, M., Chiba, T., Shimizu, S., Kawamura, Y., Nakashima, H., Nakamura, T., Takada, Y., Oikawa, Y., Takada, T., Nakaoka, H., Abe, J., Inoue, H., Wakai, K., Kawai, S., Guang, Y., Nakagawa, H., Ito, T. and Shinomiya, N. 2014. ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload. Scientific Reports, 4: 1–5.

Pacher, P., Nivorozhkin, A. and Szabo, C. 2006. Therapeutic Effects of Xanthine Oxidase Inhibitors : Human Physiology, 58(1): 87–114.

Stockert, A. L., Shinde, S. S., Anderson, R. F. and Hille, R. 2002. The reaction mechanism of xanthine oxidase: Evidence for two-electron chemistry rather than sequential one-electron steps. Journal of the American Chemical Society, 124(49): 14554–14555.

Tao, Z., Dong, B., Teng, Z. and Zhao, Y. 2020. The Classification of Enzymes by Deep Learning. IEEE Access, 8: 89802–89811.

Todorov, L., Saso, L., Benarous, K., Traykova, M., Linani, A. and Kostova, I. 2021. Synthesis, Structure and Impact of 5-Aminoorotic Acid and Its Complexes with Lanthanum (III) and Gallium (III) on the Activity of Xanthine Oxidase. Molecules, 26(15): 4503.

Tsujii, A. and Nishino, T. 2008. Mechanism of transition from xanthine dehydrogenase to xanthine oxidase: Effect of guanidine-HCL or urea on the activity. Nucleosides, Nucleotides and Nucleic Acids, 27(6–7): 881–887.

Türker, F., Noma, S. A. A., Aktaş, A., Al-Khafaji, K., Taşkın Tok, T., Ateş, B. and Gök, Y. 2020. The (NHC)PdBr2(2-aminopyridine) complexes: synthesis, characterization, molecular docking study, and inhibitor effects on the human serum carbonic anhydrase and serum bovine xanthine oxidase. Monatshefte Fur Chemie, 151(10): 1557–1567.

Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J. and Telser, J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. Molecular and Cellular Biochemistry, 266(1): 37–56.

Wood, E. J. 1996. Harper’s biochemistry 24th edition. In Biochemical Education, 2: 4.

You, Z. L., Shi, D. H., Xu, C., Zhang, Q. and Zhu, H. L. 2008. Schiff base transition metal complexes as novel inhibitors of xanthine oxidase. European Journal of Medicinal Chemistry, 43(4): 862–871.