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THE INVESTIGATION OF THE INHIBITION EFFECTS OF METAL COMPLEXES OF SCHIFF BASES OF SOME SULFA DRUGS ON 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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GHAZWAN JASSAM MOHAMMED MOHAMMED

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By Ghazwan Jassam Mohammed MOHAMMED

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

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ABSTRACT

THE INVESTIGATION OF THE INHIBITION EFFECTS OF METAL COMPLEXES OF SCHIFF BASES OF SOME SULFA DRUGS ON 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

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In particular, this study is interested in the 6PGD enzyme. In the absence of 6PGD, cancer cells die and cannot propagate. There is no correlation between a 6PGD deficiency of less than 1% and either the development or progression of cancer. Cell development is negatively affected by the inhibition of 6PGD, which controls cell growth and division. Cancer and the glucose metabolic pathway. Cancer cells need 6PGD for the synthesis of NADPH and DNA. When SIRT2 is activated, it blocks the production of NADPH and slows down DNA synthesis. Oncogenic alterations in Ras, Src, and PI3K/AKT all lead to increased 6PGD activity in cancer cells. The 6PGD gene has been linked to glioma, lung, and ovarian cancer (ROS). The 6PGD gene affects the effectiveness of therapy. BCG expression is associated with a poor outcome in bladder cancer. Treatment response and survival time for gliomas may be predicted using 6PGD. We used spectrophotometric techniques to examine the impact of several Schiff bases and their complexes on 6PGD activity in vitro. The program Molegro Virtual Docker was also used to assess probable attachment patterns.

2023, 46 pages

Keywords: Inhibition, 6PGD, Sulfa drugs, Activity, Metal complexes

ÖZET

BAZI SÜLFA İLAÇLARININ SCHİFF BAZLARININ METAL KOMPLEKSLERİNİN 6-FOSFOGLUKONAT DEHİdrojenaz AKTİVİTESİ ÜZERİNDEKİ İNHİBİSİYON ETKİSİNİN ARAŞTIRILMASI

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Bu çalışma özellikle 6PGD enzimi ile ilgilenmektedir. 6PGD'nin yokluğunda kanser hücreleri ölür ve çoğalamaz. %1'den az 6PGD eksikliği ile kanserin gelişimi veya ilerlemesi arasında bir ilişki yoktur. Hücre gelişimi, hücre büyümesini ve bölünmesini kontrol eden 6PGD'nin inhibisyonundan olumsuz etkilenir. Kanser ve glikoz metabolik yolu. Kanser hücreleri, NADPH ve DNA sentezi için 6PGD'ye ihtiyaç duyar. SIRT2 aktive edildiğinde NADPH üretimini bloke eder ve DNA sentezini yavaşlatır. Ras, Src ve PI3K/AKT'deki onkojenik değişikliklerin tümü, kanser hücrelerinde 6PGD aktivitesinin artmasına neden olur. 6PGD geni glioma, akciğer ve yumurtalık kanseri (ROS) ile ilişkilendirilmiştir. 6PGD geni, tedavinin etkinliğini etkiler. BCG ekspresyonu, mesane kanserinde kötü bir sonuçla ilişkilidir. 6PGD kullanılarak gliomalar için tedavi yanıtı ve hayatta kalma süresi tahmin edilebilir. Birkaç Schiff bazının ve bunların komplekslerinin in vitro 6PGD aktivitesi üzerindeki etkisini incelemek için spektrofotometrik teknikler kullandık. Molegro Virtual Docker programı, olası bağlanma modellerini değerlendirmek için de kullanıldı.

2023, 46 sayfa

Anahtar Kelimeler: İnhibisyon, 6PGD, Sülfa ilaçları, Aktivite, Metal kompleksleri

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Ghazwan Jassam Mohammed MOHAMMED

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CONTENTS

LIST OF SYMBOLS

mmol	Millimoles
mL	Milliliter
M	Molality
mg	Milligram
µL	Microliter

LIST OF ABBREVIATIONS

DHEA	Dehydroepiandrosteron
DNA	Deoxyribonucleic acid
FADH2	Flavin adenine dinucleotide
6PGD	Dehydrogenase for glucose-6-phosphate
H6PD	Hexose-6-Phosphate Dehydrogenase
HSPB1	Heat Shock Protein Family B (Small) Member 1
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PI3K	Phosphoinositide 3-kinase
PPP	The phosphate route
R5P	Ribose-5-phosphate
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Ru5P	Ribose-5-phosphate
SIRT2	Silent information regulator 2
SiRNA	Small interfering RNA

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1. INTRODUCTION

About 400 million people have glucose 6-phosphate dehydrogenase (6PGD) deficiency, an X-linked genetic illness. Males and females that are homozygous for the trait show full expression. Patients with 6PGD deficiency are more susceptible to hemolysis when treated with certain medicines (including primaquine, aspirin, sulfonamides, and others) because these drugs promote oxidation of hemoglobin and the red blood cell membrane. The NADPH in red blood cells is produced exclusively through the penthose phosphate metabolic pathway, which is catalyzed by 6PGD (Beutler 1971). The most crucial function of NADPH in erythrocytes is the regeneration of reduced glutathione, which protects hemoglobin from denaturation, maintains the structural integrity of sulfhydryl groups in the red blood cell membrane, and detoxifies peroxides and oxygen free radicals (Altikat *et al.* 2002). The body's natural supply of NADPH drops when 6PGD is absent. 6PGD deficiency is often encountered in African, Mediterranean, Middle East and Far East countries and their lineages with a frequency ranging from 5% to 40% (Laurence *et al.* 1997). In Turkey, incidences of this condition have been discovered among the Çukurova Region and Baºkale district of Van, and its greatest occurrence was recorded in the Jewish Kurd community (62% of males). Halothane and isoflurane are frequently used inhalation anasthetics throughout the globe. What's more, isoflurane is the best medicine available right now. Sevoflurane has just been introduced to use as inhalation anesthetic (Rang *et al.* 2003). Ketamine, an intravenous anesthetic that is a phencyclidine derivative, could be all that's needed for brief procedures like diagnostic and small surgical interventions (Laurence *et al.* 1997). Both diazepam and midazolam (two types of the benzodiazepine family) are used in anesthesia as hypnotics and sedatives, either as premedication or intraoperative sedation to achieve a state of balanced anesthesia (Trevor *et al.* 1989). Medium acting local anesthetic prilocaine is used for regional anesthesia (Rang *et al.* 2003). Many medications' effects on 6PGD enzyme activity have been studied before (Beutler 1995). The abo ve-mentioned anesthetics are commonly utilized in clini cal practice but evidence on their impact on 6PGD activ ity inadequate. Therefore, we chose to explore the in vitro effects of

halothane, isoflurane, keta-mine, sevoflurane, prilocaine, diazepam and mida zolam on 6PGD activity in human red blood cells.

As competitive inhibitors of p-aminobenzoate, sulfa medications are well-known folate synthesis inhibitors (pABA). Because of how well they imitate pABA, they may also bind to 2-amino-4-hydroxy-6-hydroxymethyl-7,8 dihydropteridine pyrophosphate to generate sulfa-dihydropteroate (sulfa-DHP), an inhibitory compound that works by competing with dihydrofolate. This indicates that sulfa medication resistance is determined by a number of factors, not only dihydropteroate synthase. In addition, the arsenal of antifolate medications may soon include new drugs based on the DHP analogues (Patel *et al.* 2003). Here, we create 6PGD cellular target engagement tests to demonstrate that SULFA DRUGS only modestly inhibit 6PGD in cells, even at high concentrations.

All living things need the redox cofactor reduced nicotinamide adenine dinucleotide phosphate (NADPH) to contribute high-energy electrons for reductive biosynthesis and antioxidant defense1. Because of their importance, these reactions rely on steady supplies of NADPH and its redox companion NADP+. Three major pathways, malic enzyme 1 (ME1), isocitrate dehydrogenase 1 (IDH1), and the oxidative pentose phosphate pathway (oxPPP) (Stanton 2012), are responsible for reducing NADP+ to NADPH in the cytoplasm of human cells. The oxPPP redirects glucose-6-phosphate from glycolysis to produce two equivalents of NADPH, one by glucose-6-phosphate dehydrogenase (6PGD), which catalyzes the first and committed step, and one by 6-phosphogluconate dehydrogenase. ME1 and IDH1 are responsible for extracting hydrides from citric acid-cycle-derived metabolites (PGD). 6PGD is expressed at high levels in immune cells and testes, but is found in all mammalian organs. Increased expression of this gene is also common in malignant growths (Nagashio *et al.* 2019). 6PGD knockout mice are genetically unable to survive (Longo *et al.* 2002). However, around one in twenty persons worldwide (Cappellini and Fiorelli 2008) have 6PGD hypomorphic alleles. Mature red blood cells (RBCs) become more susceptible to oxidative stress after acquiring these alterations, but they also provide protection against malaria. Lack of mitochondria in RBCs may explain why they are more susceptible to

mutant 6PGD, since mitochondria are responsible for producing the substrates of ME1 and IDH1. Or, it might be because RBCs don't have nuclei and can't produce a new copy of the mutant 6PGD protein as they mature. There has been little research on 6PGD's role in non-brain tissues. Recently, we used a genetic method to demonstrate that 6PGD-deficient cancer cell lines may sustain NADPH pools and grow thanks to compensatory ME1 and/or IDH1 flux (Chen *et al.* 2019). It's still unclear whether or whether cells that haven't been changed have the same degree of adaptability. Inhibitors of metabolic enzymes that are both potent and selective may help researchers learn more about their work. Many 6PGD inhibitors, including the steroid derivative dehydroepiandosterone (DHEA), have been characterized as tiny molecules (Mele *et al.* 2018). DHEA, which was discovered in 1960, binds mammalian 6PGD in an uncompetitive manner relative to both reaction substrates (Marks and Banks 1960). Since then, hundreds of studies have employed DHEA and its derivatives as 6PGD inhibitors, finding that they have antiproliferative activity (Girón *et al.* 2009) across a wide range of in vitro and in vivo cancer contexts. These indicators of cellular function are indirect, however, and it has been hypothesized that DHEA's effects may not be solely due to its suppression of 6PGD (Girón *et al.* 2009). It is essential to use assays that specifically monitor the reaction of interest in order to accurately evaluate cellular target engagement (Ducker *et al.* 2017). Because NADPH is created by several routes (where suppression of one might be hidden by compensatory production by others), it can be especially difficult to build assays that monitor NADPH-producing processes.

Pentose phosphate metabolic pathway enzymes that create NADPH include 6-phosphate glucose dehydrogenase (6- phosphate-D-glucose-NADP oxidoreductase, decarboxylating, EC 1.1.1.49; 6PGD) and 6-phospho gluconate dehydrogenase (6-phospho-D-gluconate-NADP oxidoreductase, EC 1.1.1.49). Many diverse biomolecules, including as fatty acids, steroids, and some amino acids, rely on NADPH as a coenzyme in their formation (Nelson and Cox 2000). Glutathione reductase (Glutathione: NADP oxidoreductase, E.C.1.8.1.7; GR) is a NADPH-dependent enzyme that, like glutathione synthetase, catalyzes the conversion of oxidized glutathione (GSSG) to the reduced form (GSH) of glutathione. As one of its many biological functions, GSH helps keep cells safe from damage caused by free radicals (Nelson and

Cox 2000). As well as being a reaction partner in the detoxification of endobiotics and xenobiotics, glutathione (GSH) is a cysteine that may be stored and transported (Meister and Anderson 1983). Deoxyribonucleotide synthesis and the maintenance of decreased sulfhydryl groups in intracellular proteins are two of its most important functions (Meister and Anderson 1983). A rise in protein-GSH mixed disulfides is positively linked with an increase in intracellular GSSG concentration due to oxidative stress (Brigelius *et al.* 1983). S-glutathiolation of Cys residues, for example, has been shown to affect the phosphatase activity of carbonic anhydrase (CA) III, suggesting that the creation of mixed disulfides of protein thiols and GSH may impact protein structure and regulation (Cabiscol and Levine 1996). Ocular hypotensive medications, such as CA inhibitors, are used to treat glaucoma because they lower intraocular pressure (IOP) by decreasing aqueous output. This powerful CA-II was originally developed pharmaceutically in the form of acetazolamide. It was hypothesized at the time that inhibiting the production of aqueous humor may be a useful strategy for decreasing intraocular pressure (IOP) in glaucoma treatment (Breinin 1957). After that time period, a few systemic sulfonamide medicines were mostly employed in the clinic as antiglaucoma medications (Mincione *et al.* 2001). The medicines' impact on the several CA isozymes found in different tissues led to a wide variety of undesirable side effects when taken orally. Two carbonic anhydrase inhibitors, dorzolamide hydrochloride and brinzolamide ophthalmic solution, are approved for use in the treatment of glaucoma, and have been used to mitigate the systemic side effects of their oral counterparts (Sugrue 2000). While each medication is an effective anti-glaucoma treatment, many patients have intolerable local side effects (Scozzafava *et al.* 2001). As a result, research into the potential side effects of sulfonamides used topically to treat glaucoma has been ongoing for some time (Casini *et al.* 2002). The safety and detrimental effects on certain enzymes linked with the use of various antiglaucoma medications may have a significant influence on treatment-oriented decisions, especially in the chronic clinical management of this illness. The purpose of this research is to examine the potential risks and benefits of currently available topical medications for the treatment of glaucoma that may be useful for the development of novel molecules.

2. LITERATURE REVIEW

In recent years, researchers have focused more on the role of metabolism in cancer development. Warburg noticed, more than 80 years ago, that tumor cells frequently use glycolysis as a source of adenosine triphosphate (ATP) rather than oxidative phosphorylation in the mitochondrial tricarboxylic acid cycle (TCA), even when oxygen levels are sufficient for this latter pathway to be employed (Koppenol *et al.* 2011). Compared to the 32 ATP molecules produced by the tricarboxylic acid (TCA) cycle, the eight ATP molecules produced by aerobic glycolysis seem very paltry. When their energy reserves are depleted, tumor cells may compensate by taking in more glucose. High-glycolytic cancers may now be evaluated in the clinic noninvasively using fluorescent glucose analogues, thanks to the accelerated rate at which glucose enters tumor cells (Jadvar *et al.* 2009). It has subsequently been apparent that tumor cells undergo a number of metabolic changes that facilitate the fast generation of ATP and provide the need for additional lipids and nucleotides. Due of the increased availability of metabolites, tumors are able to proliferate unchecked and to adapt to novel surroundings. The pentose phosphate pathway (PPP) is an important metabolic route (Figure 2.1).

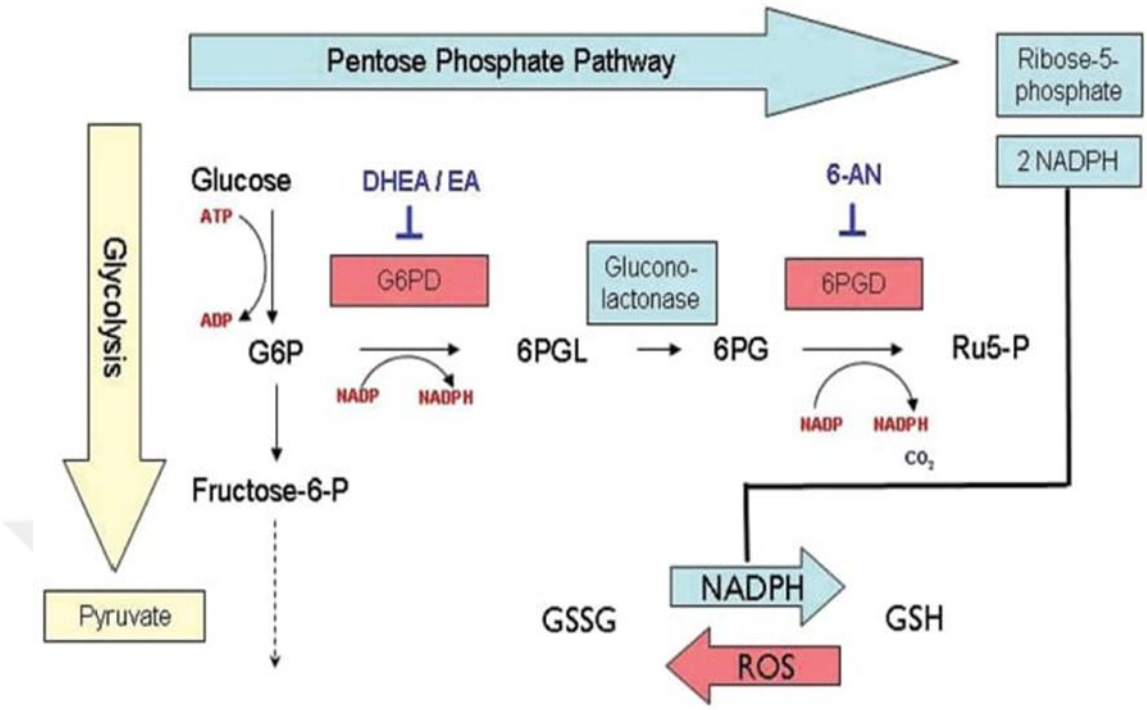

Figure 2.1 G6P is where glucose is oxidative metabolism diverges from glycolysis (Jadvar *et al.* 2009)

NADPH keeps GSH decreased. With ROS, GSH becomes GSSG. Nucleotide synthesis requires Ru5-P and ribose 5-phosphate. As glucose 6-phosphate dehydrogenase (6PGD) is rate-limiting for 6-phosphogluconate (6PG) synthesis, inhibiting 6PGD with DHEA and EA will reduce PPP flow and 6PG buildup. 6-aminonicotinamide blocks 6PGD metabolism (6-AN). At the point where glucose 6-phosphate is produced, the PPP branches off from glycolysis and into an early oxidative arm (G6P). This substrate is used in the production of ribulose 5-phosphate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Ru5-P). Many enzymes involved in macromolecular biosynthesis need NADPH as a cofactor, whereas nucleotide synthesis requires Ru5-P. The enzyme 6PGD, which converts G6P to 6-phosphogluconolactone (6PGL) while simultaneously producing NADPH, is the rate-limiting enzyme in the PPP. NADPH is employed in the defense of cells against reactive oxygen species (ROS), which are produced by rapidly replicating cells and may damage various macromolecules. Glutathione and thioredoxin are two components of an antioxidant defense system that

eliminate reactive oxygen species (ROS). In the last decade, animal models and tumor cell lines that have mutations or are null for 6PGD have allowed researchers to better understand the role of 6PGD in cancer. Tumors may develop in 6PGD overexpressing cell lines in naked mice, and these cells have an unusual appearance (Kuo *et al.* 2000). Tumor cells deficient in 6PGD, on the other hand, proliferate more slowly and have accelerated apoptosis (Li *et al.* 2009). Human fibroblasts without 6PGD are more sensitive to reactive oxygen species (ROS) and radiation, while Chinese hamster ovary cells lacking 6PGD are more sensitive to radiation (Cosentino *et al.* 2011). Studies in laryngeal and gastric cancer suggest that tumors enhance 6PGD activity, and that this links to a poor clinical outcome (Wang *et al.* 2012). Based on these findings, we set out to create a very effective inhibitor of 6PGD. If paired with radiation, it is thought that this inhibitor might weaken tumor cells' resistance to ROS and hasten their demise (Cabello *et al.* 2007). There are currently no medications available that specifically block 6PGD; nevertheless, a few small compounds with micromolar action have been identified. Dehydroepiandrosterone 1 (DHEA) and epiandrosterone 2 (EA) are two examples of steroids that have been shown to limit enzyme activity in a non-competitive manner (Gupte 2008). Numerous papers documenting the SAR of DHEA and other steroids followed its discovery (Belovsky *et al.* 1974), and more recent efforts to enhance activity have included synthetic modification (Gupta *et al.* 2011) and investigation of electrostatic potential maps (Charlton and Thomson 1994). DHEA 1 and EA 2's 6PGD inhibitory action was enhanced by a 16-bromo substituent (Gupta *et al.* 2011), although there is still room for improvement. Some studies have linked the chemopreventive and antiproliferative effects of DHEA, a common steroid in human blood, to its inhibitory 6PGD activity (Feo *et al.* 2000). However, additional mechanisms related to DHEA or its metabolites have also been invoked to explain these effects. These results emphasize the need of locating a more effective and selective 6PGD inhibitor that is less metabolically labile, as well as the possibility of off-target enzyme activity when employing DHEA. The purpose of this research was to locate a unique and powerful steroid inhibitor of 6PGD that would be easier to work with than DHEA while studying the function of 6PGD regulation in cells. If a more powerful and selective molecule existed, we could determine whether or not 6PGD inhibition really increases tumor cell susceptibility to oxidative stress and whether or not such an

inhibitor would potentially have therapeutic use in the treatment of cancer (Cheng *et al.* 2011).

It is well established that cancer cells have a very dynamic glucose metabolism. Proliferating cancer cells have developed several means of inducing 6PGD to meet their needs for NADPH+ production and nucleic acid synthesis. HSPB1 boosted NADPH and nucleic acid synthesis by activating 6PGD through SIRT2. Others increase 6PGD activity in tumor cells by activating Ras, Src, or PI3K/AKT pathways (Ye *et al.* 2016). A growing body of data indicates that elevated 6PGD expression drives tumor cell proliferation, diffusion, and migration, enhances NADPH+ generation, and decreases reactive oxygen species in leukemia, lung, ovarian, renal, and glioma malignancies (ROS). Different forms of 6PGD expression lead to different disease prognoses (Zhang *et al.* 2017). Several studies have linked the expression of 6PGD as well as the up-regulation of 6PGD to a poor prognosis for patients with bladder cancer. Computational studies have shown that 6PGD may be utilized to predict glioma risk, prognosis, and treatment responsiveness. In individuals with lung cancer, high 6PGD levels were associated with a poor prognosis that was not due to small cell disease. It has been shown that elevated 6PGD levels confer drug resistance on a wide variety of cancer cells. Increased expression of 6PGD by ID1 promotes growth of hepatocellular carcinoma and resistance to oxaliplatin (Yin *et al.* 2017). Reversal of paclitaxel resistance in ovarian cancer caused by 6PGD deficiency is caused by a decrease in GSTP1 (Feng and Sun 2020). Since 6PGD mediates and controls such a large number of ailments, research into and development of 6PGD inhibitors is warranted. DHEA is an endogenous steroid hormone, therefore it is swiftly broken down in vivo while being a potent in vitro 6PGD inhibitor. The majority of ongoing research involves using it as a model for 6PGD inhibitor development. Natural chemical polydatin may control 6PGD in vivo, leading to reduced oxidative stress. An essential step in the evolution of 6PGD inhibitors was attained with the identification of a nonsteroidal aminoquinazolinone molecule that shows promise as a reversible and non-competitive 6PGD inhibitor. More novel inhibitors might hasten the development of cancer that has been engineered to target the 6PGD therapy (Tiwari 2017). This high-throughput screening approach may also be used to analyze the activity of metabolic enzymes, thus it's not only useful for

finding inhibitors. The hypothesis behind these preliminary examinations is that 6PGD may transform G6P into 6-phospho-Dglucono-1, 5-lactone, and NADPH+. After stimulation of NADPH with light with a wavelength of 340 nm, fluorescence intensity assays measure fluorescence emission at 450 nm per minute (Haeussler *et al.* 2019).

2.1 The Function of 6PGD in Cell Growth and Division

The "housekeeping" function that 6PGD plays regulates growth and development. It has been shown that alterations in 6PGD activity or a faulty PPP may have deleterious effects on cell proliferation (Lin *et al.*, 2015) as well as embryonic and postnatal development (Yang *et al.* 2019). Amplification of either the pyruvate phosphorylation pathway or the glyoxylate cycle abnormally contributes to carcinogenesis (Zhang *et al.* 2019). Rapidly proliferating cancer cells have adapted a variety of mechanisms to activate 6PGD in order to satisfy their biological needs for NADPH generation, fatty acid and nucleic acid synthesis. Activation of Ras, Src, and PI3K/Akt, three pro-oncogenic pathways, increases 6PGD activity (Santana *et al.* 2018). Sirtuin 2, a deacetylase encoded by the SIRT2 gene, deacetylates 6PGD to elevate NADPH+ production and leukemia cell proliferation (Xu *et al.* 2016). Increasing the interaction between 6PGD and SIRT2 promotes deacetylation and activation of 6PGD (HSP27 or HSPB1). By activating 6PGD through SIRT2, HSPB1 keeps NADPH and pentose synthesis up in glioma cells (Ye *et al.* 2016). SIRT5 promotes NADPH production by increasing the desuccinylation and deglutarylation of isocitrate dehydrogenase 2 and glutamate-6-phosphate dehydrogenase (6PGD) (Zhou *et al.* 2016). In addition to STAT, Wnt/catenin, AMP-activated protein kinase, and p21-activated kinases, the 6PGD state is involved in several pathways that affect cancer cell survival (PAK) Human genetics has been intrigued by some genetic systems, either because they show variability across groups and therefore seem to be significant to human evolution or because they are linked to disease symptoms. While it is more common to study biological systems in microbes or experimental animals and then extrapolate to humans, there are occasions when this species is particularly well-suited to direct investigation, at which point characteristics of general relevance become apparent. All of these features may be seen in glucose-6-phosphate dehydrogenase (6PGD). The enzyme 6PGD is present in every

known living creature and every kind of tissue, suggesting that it has been around for a very long time. 6PGD's metabolic function has been well defined. On the one hand, it catalyzes the initial step in the biosynthesis of pentose, a substrate for all nucleotide coenzymes and a precursor to nucleic acids. Alternatively, it supplies NADPH, which is needed for many different biosynthetic and detoxifying processes. Also, it may serve as an alternate route for glucose use in certain species and conditions. As such, it is justifiable to classify it as a common and necessary enzyme for housekeeping.

2.2 This 6PGD Gene Causes Cancer

When caspases are triggered, the mitochondrial outer membrane is fully breached, or PS (polysaccharide) is exposed, dying cells send out a "eat me" signal (Kroemer *et al.* 2009). The concept of a cell death limiting point has yet to be fully addressed because of the possibility of many types of cell death occurring concurrently. RCD, ACCD, necroptosis, and necroptosis are all distinct forms of cellular death that need certain conditions to take place (Galluzzi *et al.* 2018). It could be easy to switch between the several cell killing methods. There are three major categories of cell death that are classified by their shape: type I (apoptosis), type II (autophagy), and type III (necrosis). Cell viability is only one of several biological processes that are compromised by a lack of 6PGD (Green *et al.* 2015). 6PGD's primary role in maintaining a steady cellular redox state is the regeneration of NADPH+. Insufficient 6PGD enzyme makes cells more vulnerable to adverse conditions. There is increased oxidative damage in the developing circulatory system of 6PGD-deficient embryos. In light of these results, it is reasonable to assume that 6PGD's antioxidant role is essential for normal growth and development (Ho *et al.* 2007).

2.3 The Role of Sulfonamides and Related Compounds in the Development of New Medications

According to many researchers, including (Gul *et al.* 2016, Gokcen *et al.* 2016), sulfonamide serves as the backbone of the family of drugs collectively referred to as sulfa medications. All molecules that contain the sulfonamide moiety (SO2NH2) are

referred to collectively as sulfonamides. As a result of their widespread biological applications, both the pharmaceutical and agricultural industries have shown keen interest in these compounds (Boztaş *et al.* 2015). Sulfonamide chemicals, where R is aromatic, heterocyclic, or aliphatic, are used in a wide variety of medications. Up to 30 different drugs are now in clinical use, and they all have a broad range of therapeutic variables such as those that inhibit cyclooxygenase 2 (COX2), antiviral, antibiotic, diuretic, and protease. Among the many diseases and disorders that these drugs are used to treat include conjunctivitis, bacillary dysentery, meningitis, streptococcal pharyngitis, trachoma, nocardiosis, and malaria (Gulçin and Taslimi 2018). Type 2 diabetes mellitus is characterized by hyperglycemia as well as by an impaired balance with both fibrinolysis and coagulation; therefore, metformin sulfonamide derivatives, for example, may have anticoagulant and antifibrinolytic properties and could be evaluated in the screening of effective pharmaceutical candidates for the treatment of Type 2 diabetes mellitus (Molyneux *et al.* 2019). Patients with hyperglycemia, metabolic acidosis, and high ketone body levels in the blood circulation may experience euglycemic episodes when their serum glucose levels are within the normal range because aldose reductase is a rate-determining enzyme in the polyol pathway and phosphorylates glucose under euglycemic conditions. The findings from the aforementioned studies have important implications for the management of type 2 diabetes (Apaydn and Török 2019). Sulfonamide derivatives, especially chalcones (also known as -unsaturated ketones), have shown promise in the laboratory (Di Carlo *et al.* 1999, Guida *et al.* 1997). They constitute a significant component of natural resources and are primary inputs for fabricated changes. Many different types of biological activity may be seen in chalcones and their synthetic analogues. In contrast to their apparent inertness, chalcones are thought to be bioactive due to the double bond conjugation of the carbonyl functional group. They are found in both trans and cis configurations and are amenable to cyclization by Michael's addition to yield flavanones. ClaiseneSchmidt condensation under homogeneous circumstances in the presence of acid or base is an integral part of many common synthetic techniques for chalcones production (Dhar 1981). For centuries, industry has relied on highly alkaline media such natural phosphates and others (Climent *et al.* 2004, Daskiewicz *et al.* 1999). There have been other additional lewis acids used (Iranpoor and Kazemi 1998, Nakano *et al.* 1987, Singh *et al.* 2014).

2.4 Sulfa Drugs

In spite of their antiquity as chemically manufactured antimicrobial agents, sulfa medicines are nevertheless often utilized to treat a broad range of microbial illnesses today. Forty years after Gene Brown discovered that sulfa medications worked by competitive inhibition of the enzyme dihydropteroate synthase (DHPS), a critical enzyme in folate production, the discovery of a second mode of their action and perhaps of resistance is unexpected. (Figure 2.2) shows the folate production route, which is found only in microbes and plants. In this system, p-aminobenzoate (pABA) condenses with 2-amino-4-hydroxy-6-hydroxymethyl-7,8 dihydropteridine pyrophosphate (DHPPP), a reaction mediated by DHPS, to create dihydropteroate (DHP). Dihydrofolate synthase (DHFS) uses DHP as a substrate to create dihydrofolate from glutamate (DHF). To create tetrahydrofolate, the enzyme dihydrofolate reductase (DHFR) reduces dihydrofolate (DHF) (THF). Biosynthetic processes involving the production of amino acids like methionine, serine, glycine, and histidine, as well as purines and thymidylates, all rely on THF and its derivatives as cofactors. Due to the lack of the folate synthesis pathway in humans, folate is a major microbial target since it is essential for proper cellular development and function (Brown *et al.* 1962).

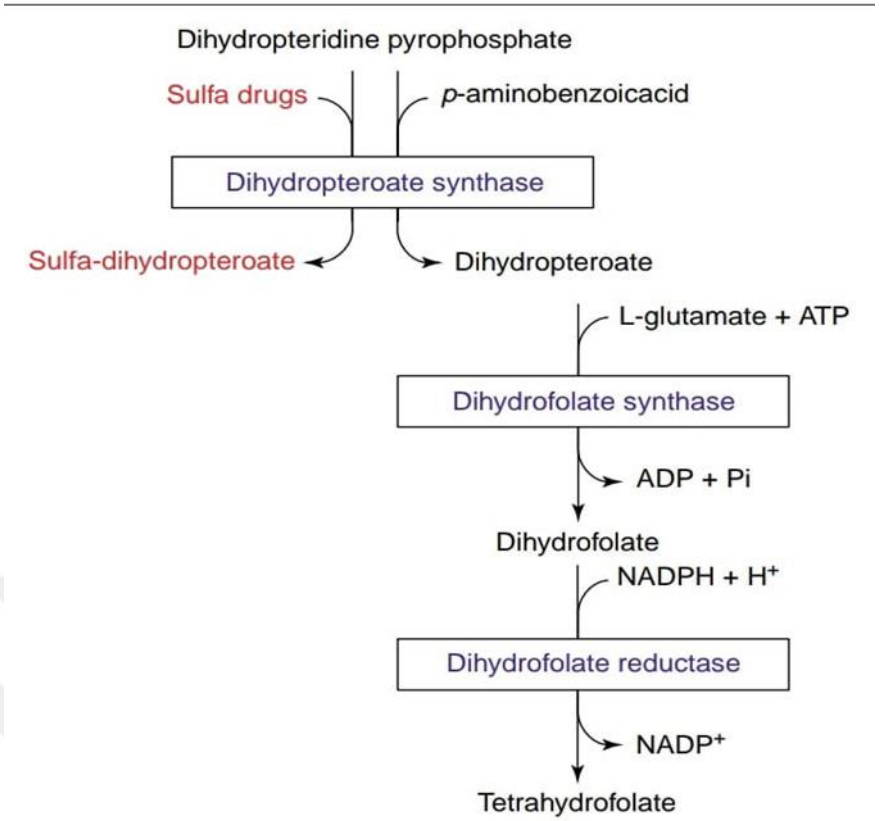

Figure 2.2 Folate biosynthesis (Brown *et al.* 1962)

Dihydropteroate synthase (DHPS) catalyzes the condensation of p-aminobenzoic acid to dihydropterin pyrophosphate to create dihydropteroate, which is converted to dihydrofolate. Dihydrofolate reductase makes tetrahydrofolate. In the presence of a sulfa medication, DHPS forms sulfa-dihydropteroate. Red shows sulfa medicines and sulfa-DHP. Blue indicates enzymes and black typical metabolites.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Regents

NADP+ (NADP+ phosphate salt) must be present in the cell in order for the cell to produce energy.

Glucosyl 6-Phosphatidyl Glycoside (mono Na salt).

Hydrogen trisulfide is a gas that may be found in the atmosphere.

3.1.2 Equipment

In the study, tools such as these were used Table 3.1.

Table 3.1 Methods similar to these were used in the research

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

3.1.3 The preparation and use of diverse solutions

Measurement methods for activities

- A Tris-HCl (pH 8.0) solution was prepared by dissolving 0.6057 grams (5 millimoles) in 90 mL of water. We used an HCl solution to get the pH level up to 8.00. Repeating the process until the last 50 mL of water were gone.

- A total of 9.1 mg of 6PGA was synthesized from 0.3 mmol of liquefied natural gas (LNG) dissolved in water. There was a need to increase the volume to 5 mL, thus water was added.

- NADP+ at a concentration of 2 mM/L To get a 0.1 mmol solution, water was added to a 7.6 mg/mmol solution of NADP+. It was just 5 mL before water was added.

- The compounds were dissolved in DMSO at a concentration of 1 mg/L, and the resulting stock solution was transferred to the test tube.

3.2 Methods

3.2.1 This approach may be used to measure enzyme activity

To reduce 6-phosphogluconate, 6-phosphogluconate dehydrogenase is required, as shown by Equation 3.1 involving NADP+. This is an enzymatic reaction, hence NADPH is a byproduct. Light with a wavelength of 340 nm is very effectively absorbed by NADPH. The enzyme's efficiency was determined by monitoring the intensity of a 340-nanometer NADPH fluorescence rise (Beutler 1971). 96-well microplates were utilized for the activity testing. Table 3.2 covers the many aspects of activity measurement.

$$6-phosphogluconate \ + \ NADP + \xrightarrow{6PGD} \ Ribose-5-phosphate \ + \ NADPH \qquad (3.1)$$

Table 3.2 Test for 6PGD Enzyme Activity

	Control cuvette	Sample cuvette
Stock solutions	Volume (µL)	Volume (µL)
1 M Tris-HCl	50	50
2 mM $NADP^+$	20	20
Pure water	100	100
Enzyme sample	10	10
10 minutes incubation		
6 mM 6PGA	-	20

3.2.2 Suppression of enzymes

In this research, it was shown that derivatives of N-benzylindole had potent effects as both enzyme inhibitors and activators. With or without an inhibitor, the substrate concentrations (6.25, 15.30, 62.5, and 90 M) in the reaction medium were G6P and 6PGA, respectively. The reaction media was filled to a volume of 1 mL, and three predetermined concentrations of N-benzoylindole derivatives were added. The half-maximal inhibitory concentration (IC50) for G6P/6PGA was determined by testing the enzyme's activity in the presence of increasing concentrations of the inhibitor. No compounds were detected, however full enzyme activity was identified. Concentrations (IC50 values) at which enzyme activity is reduced by 50% were determined by plotting the percentage of enzyme activity against the concentration of the chemical.

In order to test whether or not flavone and flovanol molecules impacted enzyme activity, they were dissolved in DMSO at a concentration of 1 mg/mL. Enzyme activity was measured using a 10-fold dilution series to compare substances of varying concentrations. It took diluting compounds with a strong inhibitory effect to bring enzyme activity down by a factor of 2. Researchers analyzed five concentrations of enzyme activity to identify the threshold at which half of the activity is present. We compared results using inhibitor-free enzyme activity absorbance as our benchmark. Percentage of activity is how the inhibitors' impact was measured. This led to the development of percent activity-[concentration] diagrams. These plots were used for IC50 determinations.

3.2.3 Molecular docking studies

We utilized PubMed to collect the 3D structures of all drugs with potential inhibitory effects. The crystal structure of the human 6PGD enzyme was obtained in a pdb file on the Protein Data Bank website (Au *et al.* 2000).

The protein has been uploaded to the Molegro Virtual Docker, and it is now ready to dock. The crystal structure of the protein was cleaned up to reveal just the ligand and no other molecules or atoms. The program received molecules (Molegro 2019). When looking for a docking site, the NADPH binding site was discovered. There were 10 docking attempts for each ligand in the algorithm. There was careful consideration of all available MolDock Scores.

The 6PGD crystal structure was used as the basis for this study (PDB code: 5UQ9). As soon as it was placed there, the NADP+ binding site was visible in the BIOVIA Discovery Studio 2017 R2 (DS) program. When the ligand attaches to the receptor, it forms a hydrogen bond with the receptor and also makes van der Waals contact and an electrostatic connection..

4. RESULTS AND DISCUSSION

Table 4.1 displays the study's findings, which were separated into two categories. The first segment, denoted by the letter (i) is the inhibitor, whereas the second, denoted by the letter (ne), has no effect. In the subsequent section of this thesis, we shall go into further depth on these issues.

Table 4.1 Distribution of findings and results of a study

No	Compound Name	IC50 (µM)	Effects
1	S1M-S1	-	ne
2	Pd(S1M-S1)2	7,56	i
3	Cu(S1M-S1)2	66,58	i
4	S2M-S1	-	ne
5	Pd(S2M-S1)2	63,24	i
6	Cu(S2M-S1)2	66,27	i
7	SIM-S3 (2OHS1-S1)	260,58	i
8	Pd(S1M-S3)2	5,12	i
9	Cu(S1M-S3)2	20,51	i
10	S2M-S3	396,08	i
11	Pd(S2M-S3)2	2,73	i
12	Cu(S2M-S3)2	49,80	i

To put it another way, the $Pd(S1M\text{-}S1)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Pd(S1M\text{-}S1)_2$ against 6PGD enzyme were seen at an IC50 value of 7,56 M Figure 4.1 and 3D interactions maps were shown in Figure 4.2. Three-dimensional mapping of enzyme interactions revealed that Lys 76, Ala 77, Gln 79 and Asn 103 formed hydrogen bonds with $Pd(S1M\text{-}S1)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.2.

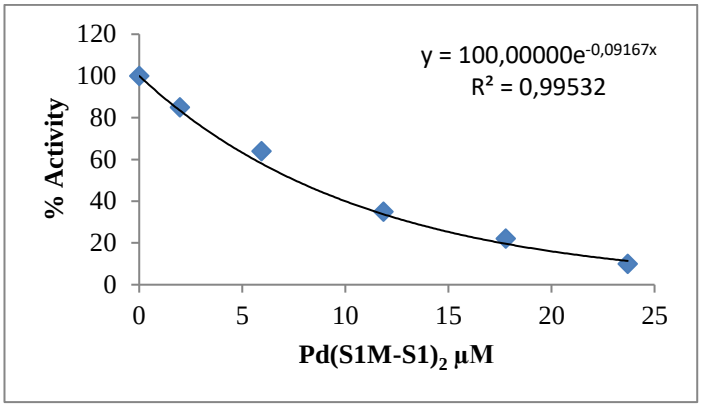

Figure 4.1 6PGD enzyme chemical activity as a fraction of total [$Pd(S1M\text{-}S1)_2$]

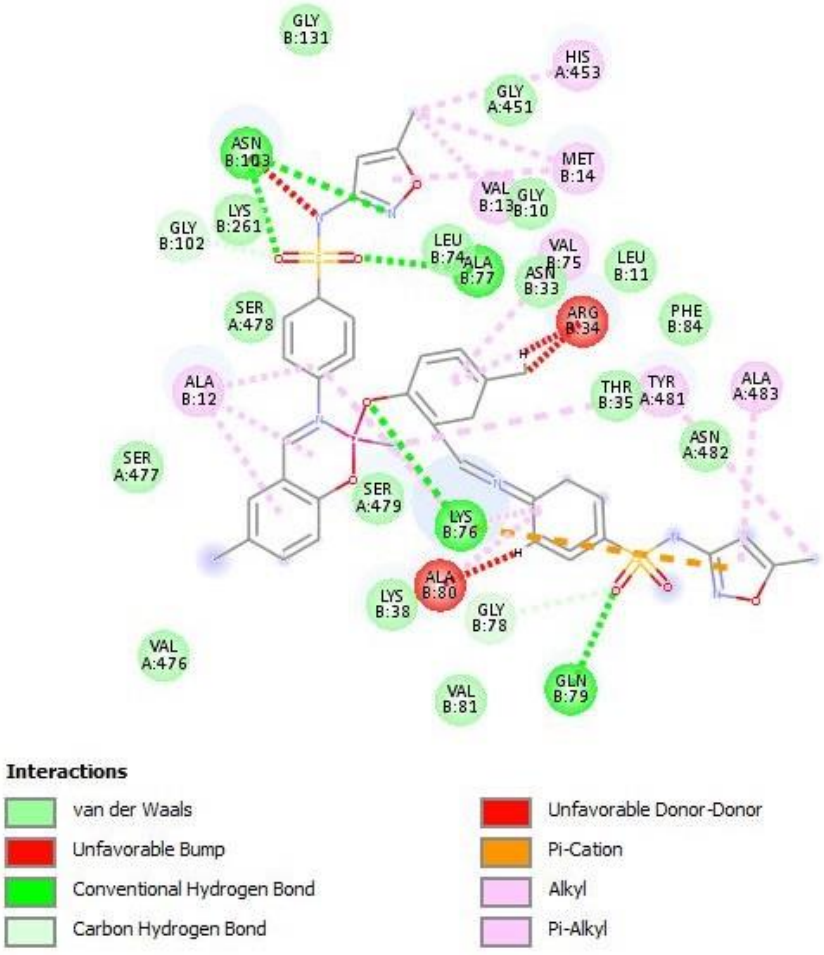

Figure 4.2 The active region of 6PGD communicates with $Pd(S1M\text{-}S1)_2$

To put it another way, the $Cu(S1M\text{-}S1)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Cu(S1M\text{-}S1)_2$ against 6PGD enzyme were seen at an IC50 value of 66,58 M Figure 4.3 and 3D interactions maps were shown in Figure 4.4. Three-

dimensional mapping of enzyme interactions revealed that Lys 76 formed hydrogen bonds with $Cu(S1M\text{-}S1)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.4.

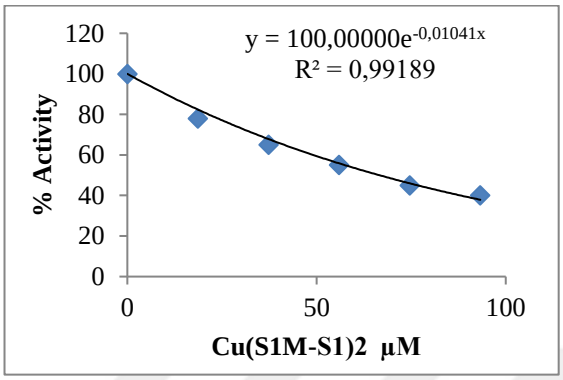

Figure 4.3 6PGD enzyme chemical activity as a fraction of total [$Cu(S1M\text{-}S1)_2$]

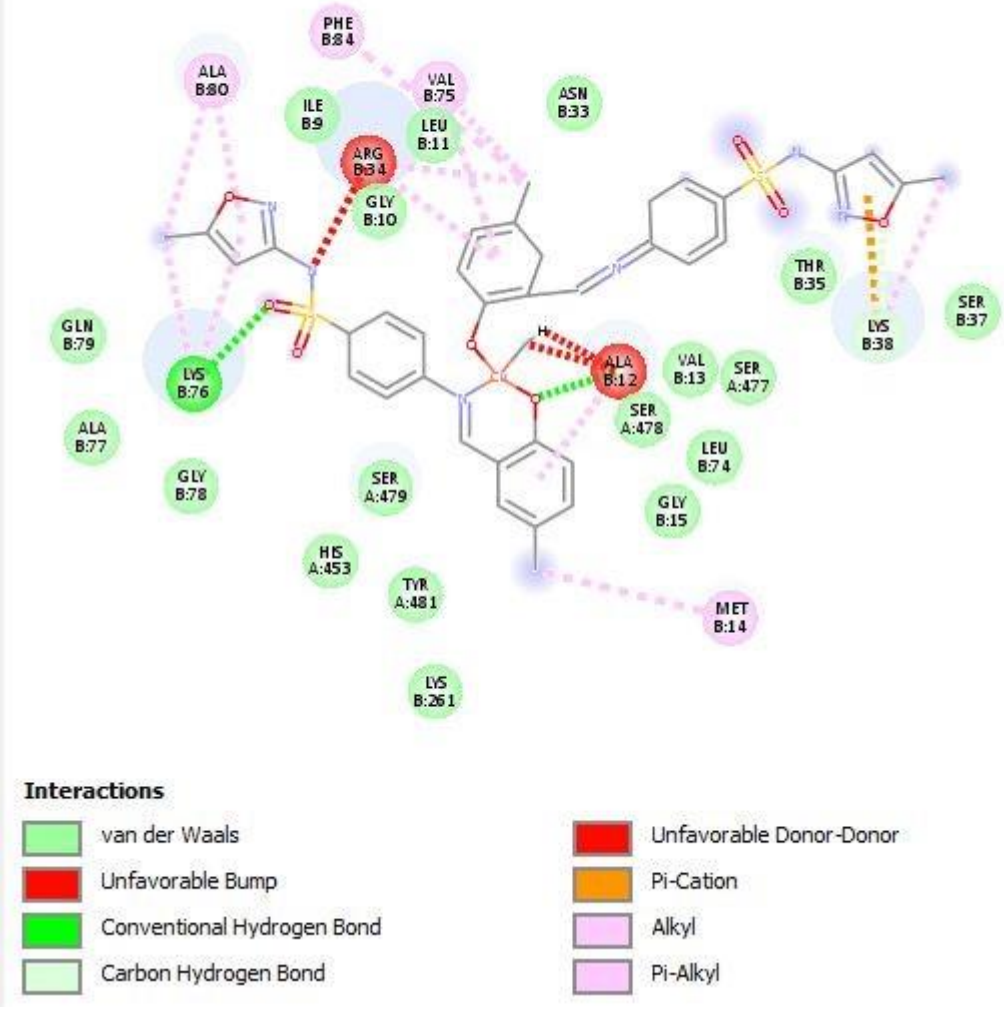

Figure 4.4 The active region of 6PGD communicates with $Cu(S1M\text{-}S1)_2$

To put it another way, the $Pd(S2M\text{-}S1)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Pd(S2M\text{-}S1)_2$ against 6PGD enzyme were seen at an IC50 value of 63,24 M Figure 4.5 and 3D interactions maps were shown in Figure 4.6. Three-dimensional mapping of enzyme interactions revealed that Asn 33, Arg 34, The 35, Lys 76 and Ser 479 formed hydrogen bonds with $Pd(S2M\text{-}S1)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.6.

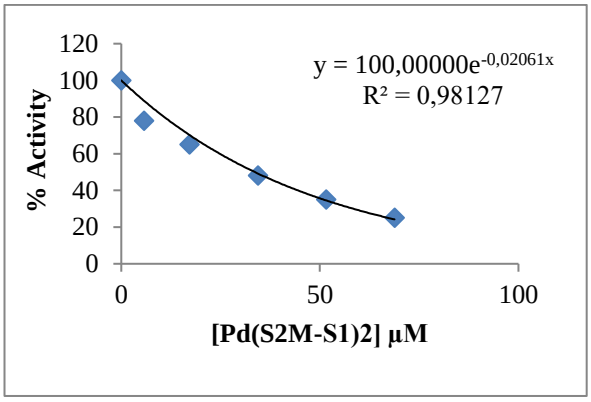

Figure 4.5 6PGD enzyme chemical activity as a fraction of total [$Pd(S2M\text{-}S1)_2$]

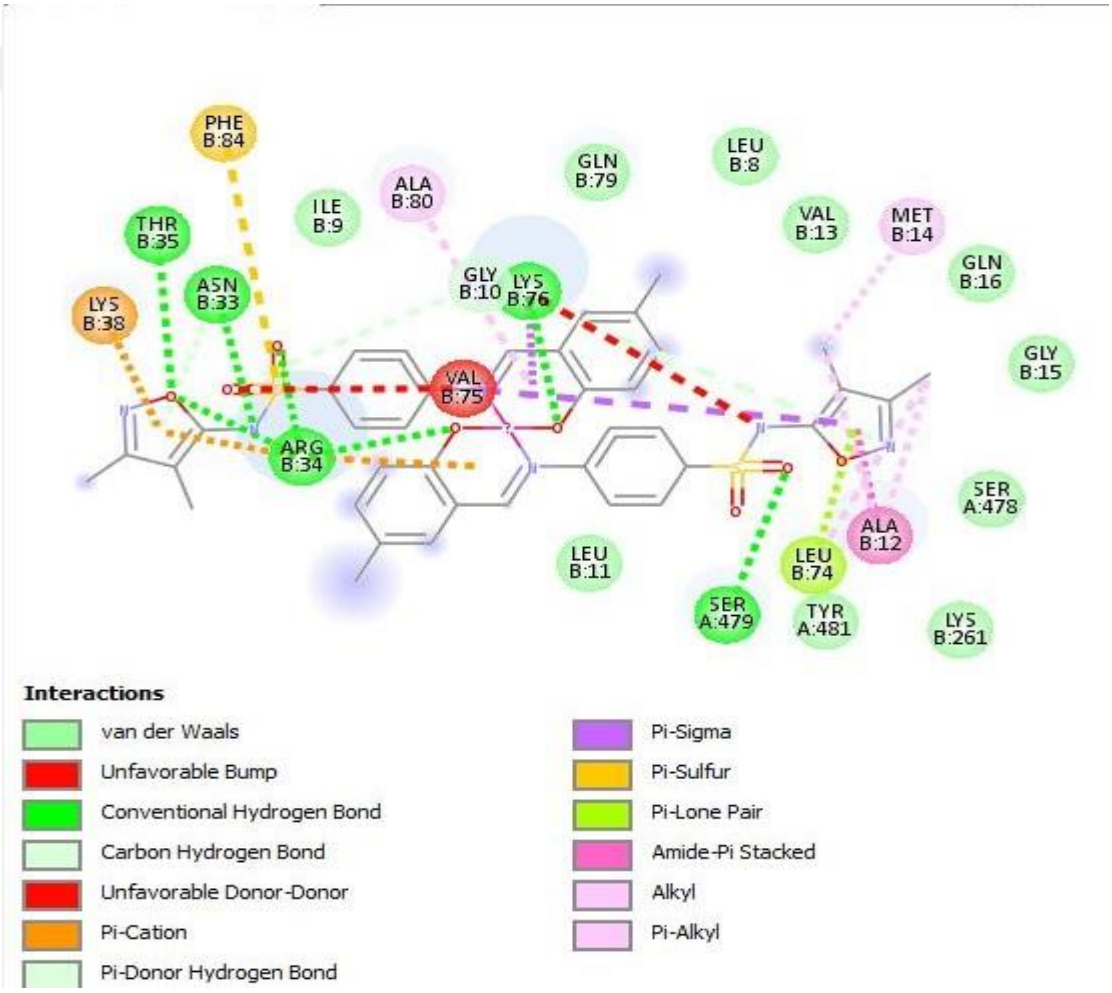

Figure 4.6 The active region of 6PGD communicates with $Pd(S2M\text{-}S1)_2$

To put it another way, the $Cu(S2M\text{-}S1)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Cu(S2M\text{-}S1)_2$ against 6PGD enzyme were seen at an IC50

value of 66,27 M Figure 4.7 and 3D interactions maps were shown in Figure 4.8. Three-dimensional mapping of enzyme interactions revealed that Ser 477 formed hydrogen bonds with $Cu(S2M\text{-}S1)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.8.

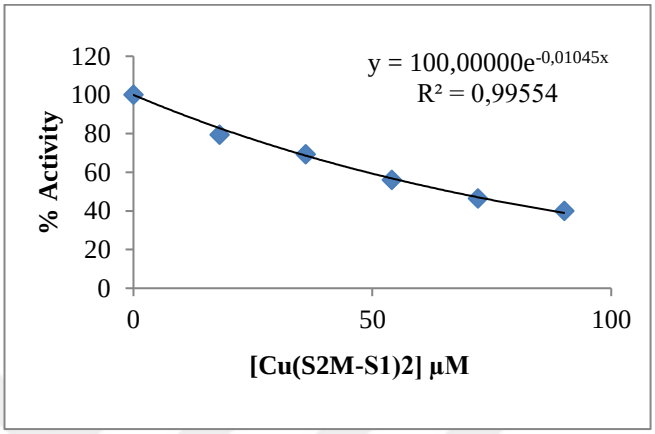

Figure 4.7 6PGD enzyme chemical activity as a fraction of total $[Cu(S2M\text{-}S1)_2]$

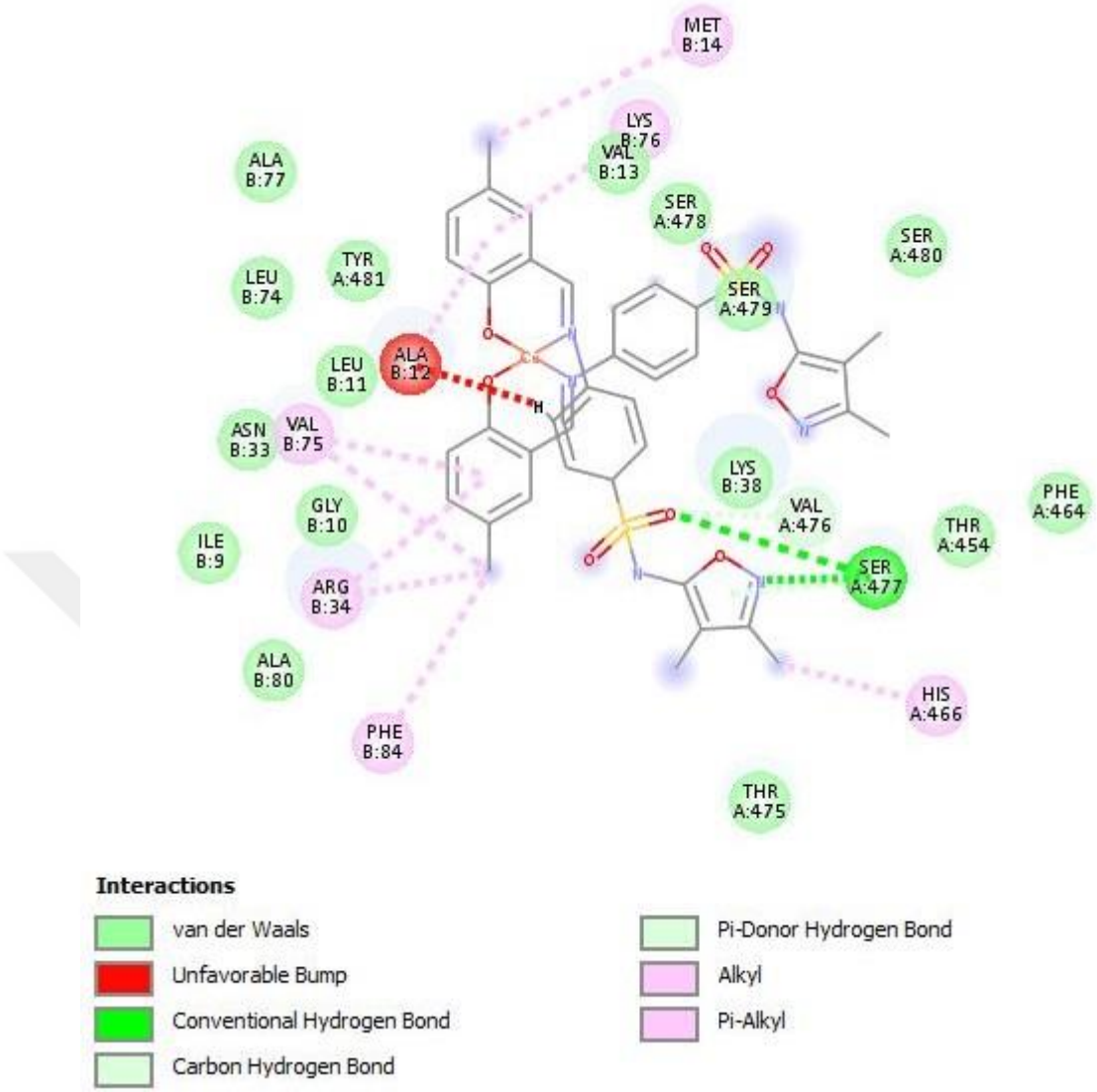

Figure 4.8 The active region of 6PGD communicates with Cu(S2M-S1)$_2$

To put it another way, the SIM-S3 (2OHS1-S1) molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of SIM-S3 (2OHS1-S1) against 6PGD enzyme were seen at an IC50 value of 260,58 M Figure 4.9 and 3D interactions maps were shown in Figure 4.10. Three-dimensional mapping of enzyme interactions revealed that Met 14, Gly 15, Leu 74, Asn 103, Lys 184 and Ser 478 formed hydrogen bonds with SIM-S3 (2OHS1-S1). Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.10.

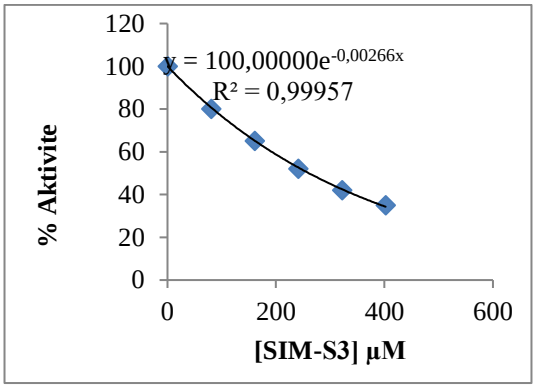

Figure 4.9 6PGD enzyme chemical activity as a fraction of total [SIM-S3 (2OHS1-S1)]

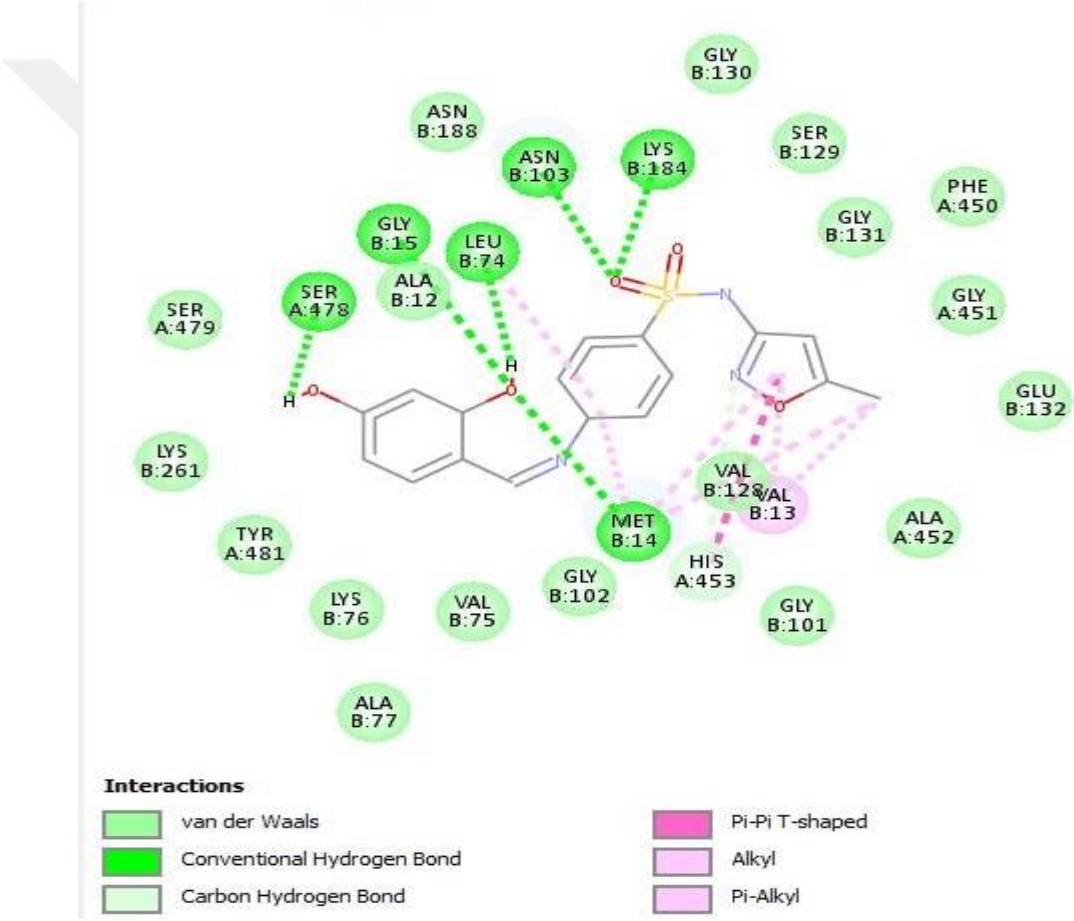

Figure 4.10 The active region of 6PGD communicates with SIM-S3 (2OHS1-S1)

To put it another way, the Pd(S1M-S3)$_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of Pd(S1M-S3)$_2$ against 6PGD enzyme were seen at an IC50 value of 5,12 M Figure 4.11 and 3D interactions maps were shown in Figure 4.12. Three-dimensional mapping of enzyme interactions revealed that Asn 33, Lrg 34 and

Leu 74 formed hydrogen bonds with $Pd(S1M\text{-}S3)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.12.

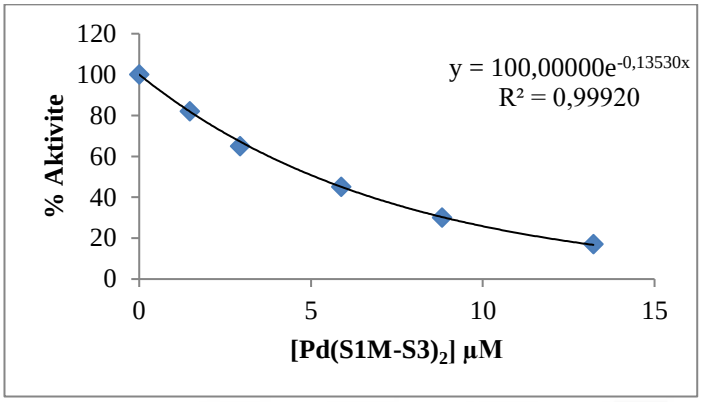

Figure 4.11 6PGD enzyme chemical activity as a fraction of total $[Pd(S1M\text{-}S3)_2]$

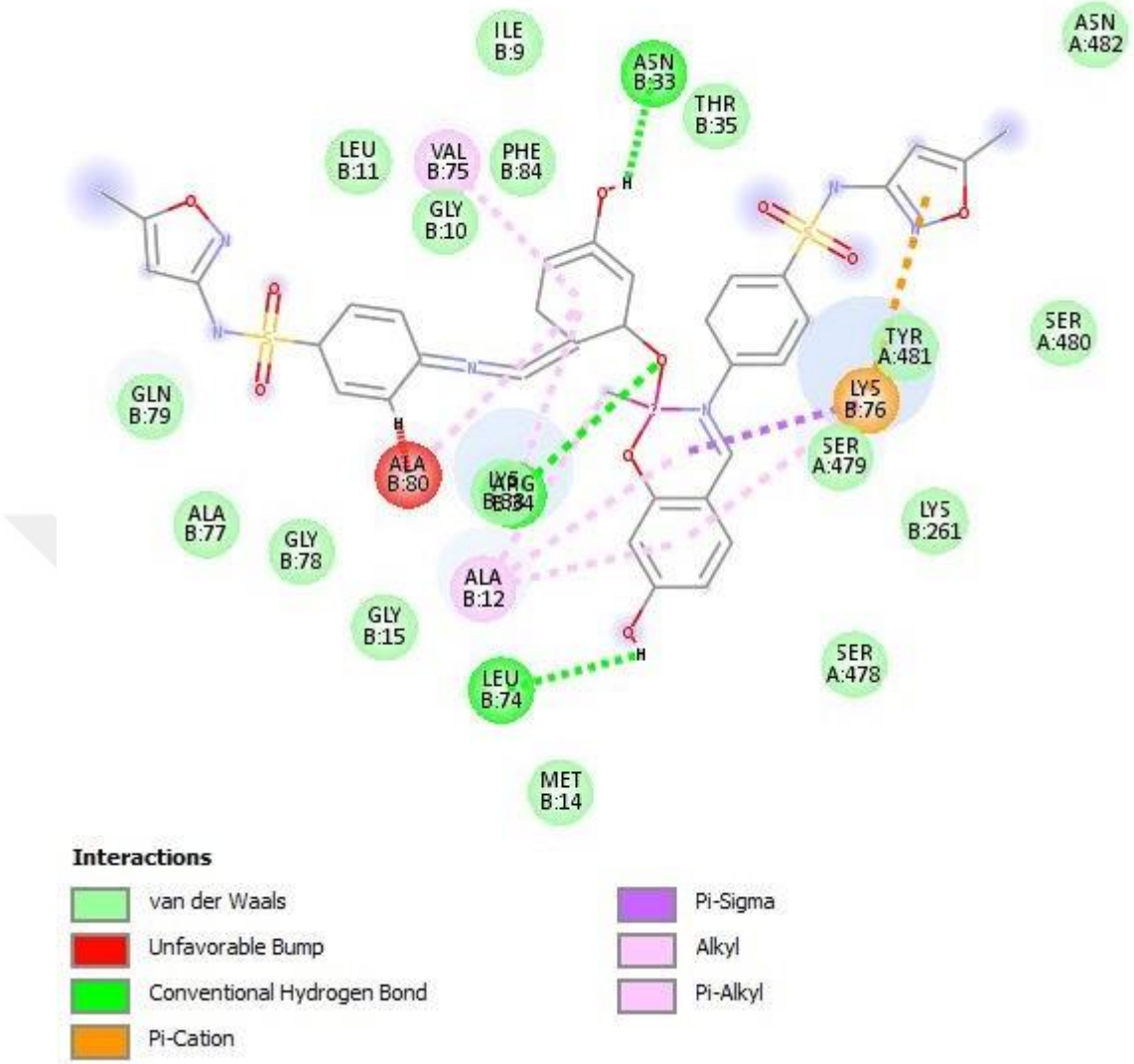

Figure 4.12 The active region of 6PGD communicates with Pd(S1M-S3)$_2$

To put it another way, the Cu(S1M-S3)$_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of Cu(S1M-S3)$_2$ against 6PGD enzyme were seen at an IC50 value of 20,51 M Figure 4.13 and 3D interactions maps were shown in Figure 4.14. Three-dimensional mapping of enzyme interactions revealed that Asn 33, Lys 76, Gln 79, Asn 103 and Lys 261 formed hydrogen bonds with Cu(S1M-S3)$_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.14.

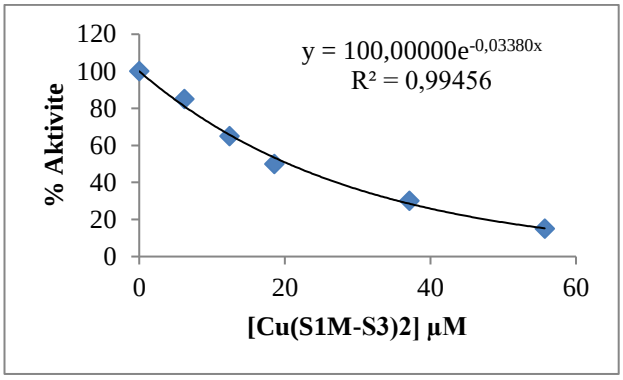

Figure 4.13 6PGD enzyme chemical activity as a fraction of total [$Cu(S1M\text{-}S3)_2$]

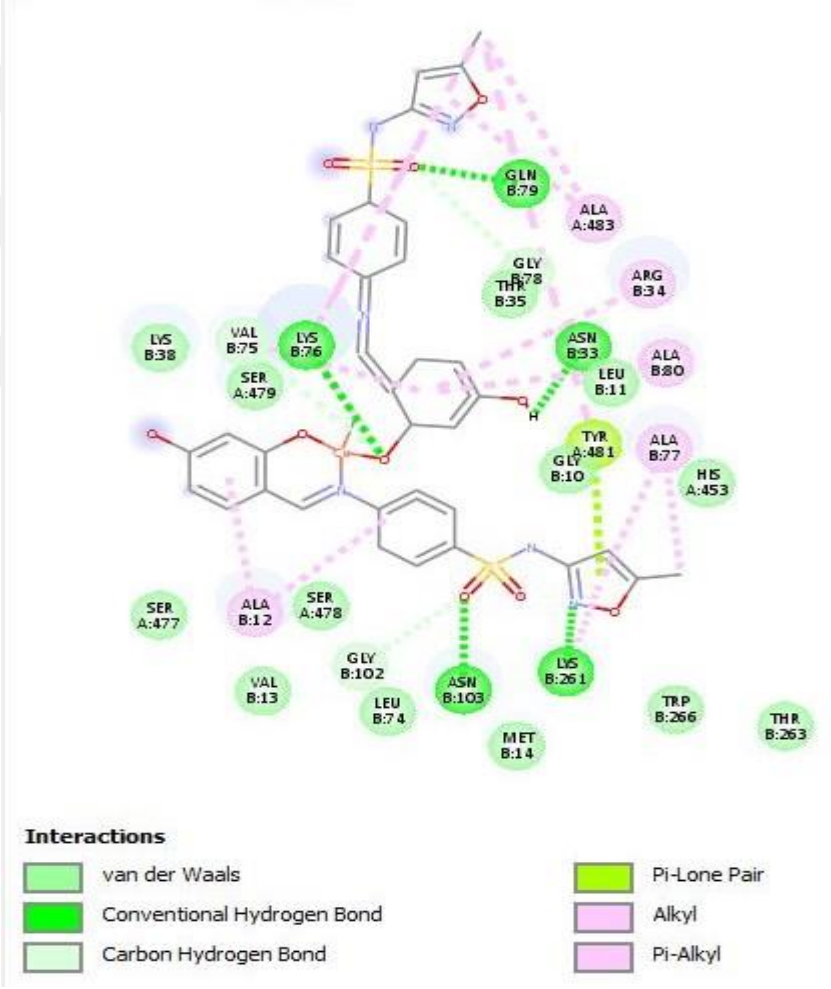

Figure 4.14 The active region of 6PGD communicates with $Cu(S1M\text{-}S3)_2$

To put it another way, the S2M-S3 molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of S2M-S3 against 6PGD enzyme were seen at an IC50 value of 396,08 M Figure 4.15 and 3D interactions maps were shown in Figure 4.16. Three-

dimensional mapping of enzyme interactions revealed that Val 75, Asn 188, Lys 261, Thr 263, Arg 288 and Arg 447 formed hydrogen bonds with S2M-S3. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.16.

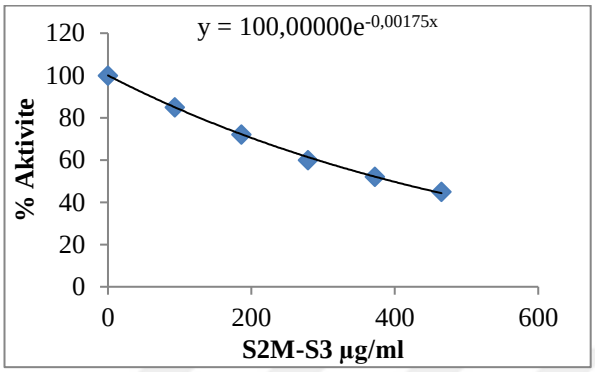

Figure 4.15 6PGD enzyme chemical activity as a fraction of total [S2M-S3]

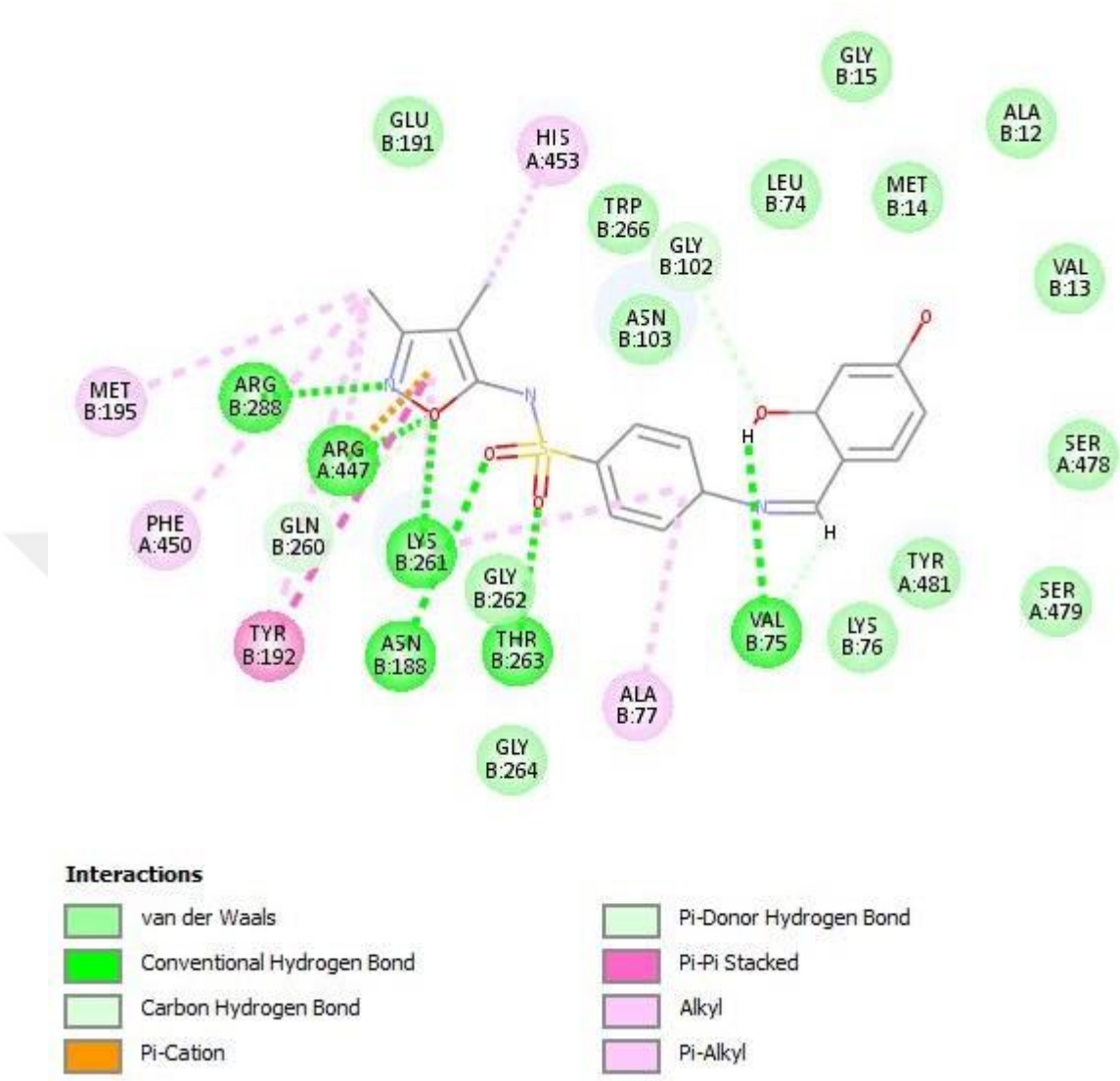

Figure 4.16 The active region of 6PGD communicates with S2M-S3

To put it another way, the $Pd(S2M\text{-}S3)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Pd(S2M\text{-}S3)_2$ against 6PGD enzyme were seen at an IC50 value of 2,73 M Figure 4.17 and 3D interactions maps were shown in Figure 4.18. Three-dimensional mapping of enzyme interactions revealed that Asn 33, Arg 34, Lys 76 and Gln 79 formed hydrogen bonds with $Pd(S2M\text{-}S3)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.18.

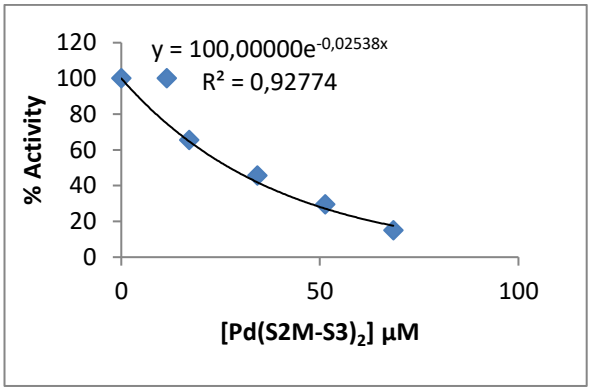

Figure 4.17 6PGD enzyme chemical activity as a fraction of total [$Pd(S2M\text{-}S3)_2$]

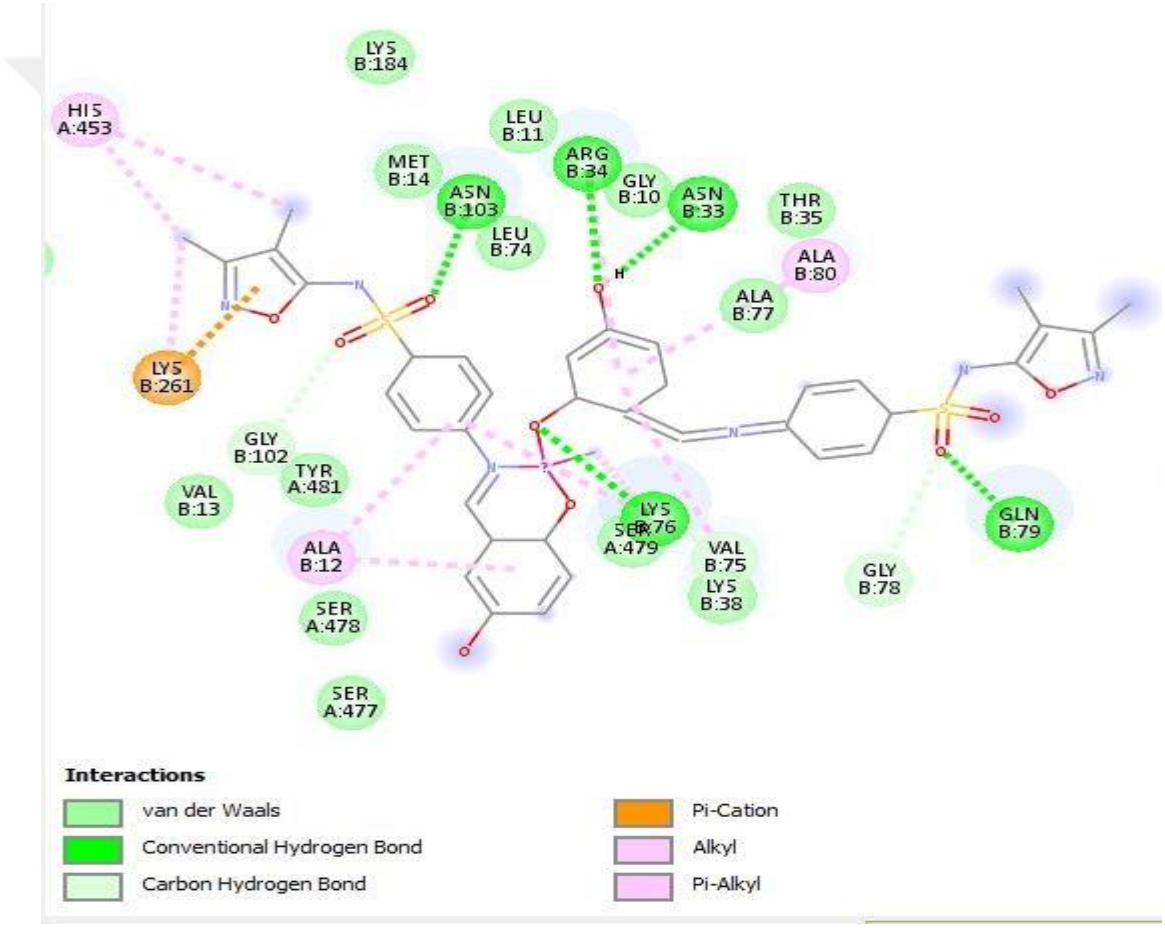

Figure 4.18 The active region of 6PGD communicates with $Pd(S2M\text{-}S3)_2$

To put it another way, the $Cu(S2M\text{-}S3)_2$ molecule acted as an inhibitor of the 6PGD enzyme. Inhibitory effects of $Cu(S2M\text{-}S3)_2$ against 6PGD enzyme were seen at an IC50 value of 49,80 M Figure 4.19 and 3D interactions maps were shown in Figure 4.20. Three-dimensional mapping of enzyme interactions revealed that Met 14, Asn 33 and

Ser477 formed hydrogen bonds with $Cu(S2M\text{-}S3)_2$. Hydrogen bonds, shown here by the thin green lines, are clearly visible in Figure 4.20.

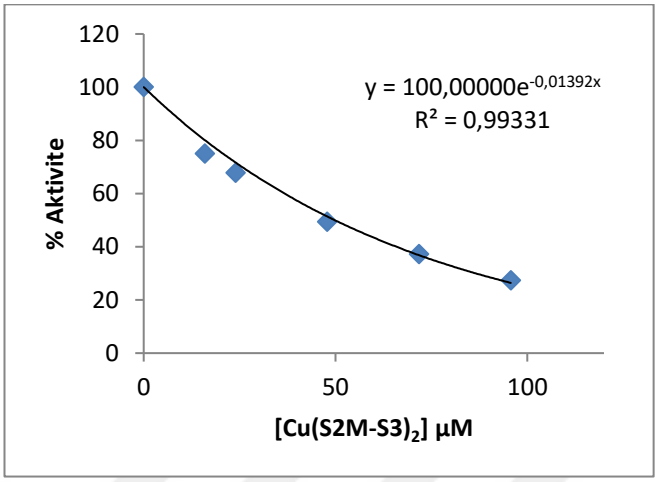

Figure 4.19 6PGD enzyme chemical activity as a fraction of total $[Cu(S2M\text{-}S3)_2]$

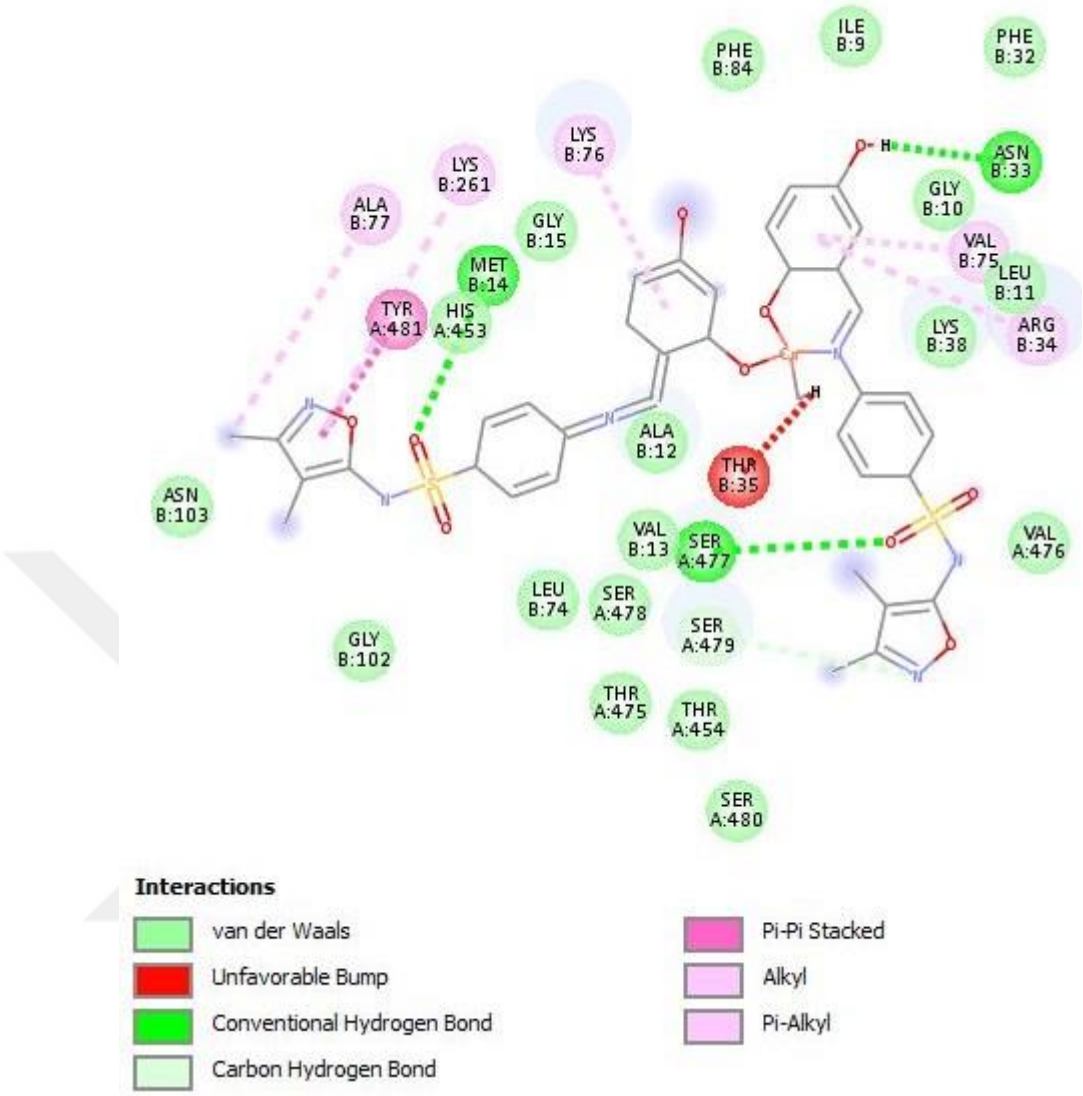

Figure 4.20 The active region of 6PGD communicates with Cu(S2M-S3)$_2$

Even after all of the necessary conditions were met, there were still several adherens around the active 6PGD residue. For every molecule, the top 12 compounds were chosen. The results of the molecular fusion experiment showed that the twelve compounds were split into an inhibitor component (represented by the letter (I)) and a non-inhibitory component (represented by the letter (ne)). Ten compounds were first tried, all of which had a noticeable impact and were structurally close to one another in the active area of 6PGD. The third and final portion contains two compounds that had no noticeable results in the lab. Inhibition of certain enzymes is a common mechanism by which even modest doses of substances may disrupt normal enzyme function and so the body's metabolism (Hochster *et al.* 1973). Some antibiotics have been shown to

block 6PGD, 6PGD, and GR enzyme activity, which may explain why they are so ineffective against bacterial infections. To provide only two examples, ampicillin suppresses 6PGD in human red cells (Ciftci *et al.* 200) and in sheep liver (Beydemir *et al.* 2002). Similarly, amikacin suppresses 6PGD in human red cells (Ciftci *et al.* 200). Amikacin, ampicillin, and netilmicin have all been shown to inhibit rat erythrocyte 6PGD (Çiftçi *et al.* 2002). For example, both ofloxacin and cefepime have been shown to inhibit chicken liver 6PGD (Erat and Sakiroglu 2007). Inhibitors of GR from sheep liver (Erat and Çİftçİ 2003) and chicken liver (Demir *et al.* 2006) include ofloxacin, levofloxacin, cefepime, and cefazolin. Because of their 1, 3, 4-thiadiazole rings, several sulfonamide derivatives are potent chemotherapeutic medicines used to treat bacterial infectious diseases (Ashour *et al.* 1990). Many of them are potent inhibitors of CA isozymes (BüLBüL *et al.* 2003), a number of different brinzolamides (1, 2, 3, 4, 5, and 6) and acetazolamide. Because of their efficacy in reducing intraocular pressure, these pharmaceuticals are a mainstay in the glaucoma therapy arsenal (Casini *et al.* 2002). The diuretic effect is produced by blocking CA isozymes in human kidney tubule cells. In addition, several different kinds of tumor cells have been shown to be sensitive to the growth inhibitory effects of aromatic sulfonamide derivatives (Supuran and Scozzafava 2000). 6PGD deficiency is the most frequent enzymatic defect in red blood cells in the globe (Weksler *et al.* 1990). When aged erythrocytes are exposed to oxidant medications or chemicals, they inevitably bleed to death. Hemolysis may cause hemoglobinuria and acute renal failure, whereas more severe 6PGD deficiency results in anemia, jaundice, and reticulocytosis. Patients undergoing surgery often have general anesthetics administered to them to ensure they are completely sedated and unresponsive to any uncomfortable stimuli. They have mostly central nervous system effects when administered systemically (Rang *et al.* 2003). Analgesia, forgetfulness, unconsciousness, suppression of sensory and autonomic reflexes, and relaxation of skeletal muscles are the typical effects of general anesthetics. Inhalation or intravenous injection are the most common routes of administration for general anesthetics. There are a number of medications that may be administered intravenously to induce or maintain anesthesia. Preoperative sedation and analgesia, intraoperative neuromuscular blocking medicines, and intravenous and inhalation anesthetics are all components of a balanced anesthetic regimen. One of the first and most popular halogenated agents was

halothane. However, its popularity is waning as alternatives such as isoflurane become more widely used. Due to their low toxicity, isoflurane and sevoflurane are the best volatile anesthetic medicines. However, this research shows that halothane, unlike isoflurane and sevoflurane, does not affect 6PGD activity. It is for this reason that halothane may be used safely on a patient with 6PGD deficiency. Furthermore, the combination of isoflurane or sevoflurane with diazepam or midazolam, which have inhibitory effects on in vitro 6PGD enzymatic activity, may exacerbate the severity of hemolysis. In order to get a more well-rounded anesthetic effect, ketamine may be substituted for midazolam or diazepam as the intravenous anesthetic of choice. When local anesthetic is necessary, prilocaine is the drug of choice. Finally, we believe that our study's findings may aid in the selection of anesthetic medicines for a patient with 6PGD deficiency. Patients with 6PGD deficiency may have a rapid deterioration in health if general anesthetic medications that suppress 6PGD are used. Drugs that block 6PGD metabolism may be necessary for people with this condition, but their dosing must be carefully regulated to limit undesirable consequences. Clinically, it is crucial to understand the impact of these medications on 6PGD activity in living organisms, hence this area of research has to be expanded upon.

5. CONCLUSIONS AND RECOMMENDATION

Cancer is linked to the 6PGD enzyme because of its crucial role in cell signaling that regulates cell growth and death. Rapidly dividing cancer cells often have problems with 6PGD activation, which has been linked to tumors and malignancies. If 6PGD can be used as a therapeutic target, existing anticancer drugs may be combined with it to overcome cancer resistance. 6PGD inhibitors, either pharmacologic or molecular, are a frequent component of many existing techniques. Inhibitors of 6PGD are created by targeting this enzyme in the body's metabolic process. It is essential to maintain redox homeostasis and protein-protein interactions to prevent rapid transformation, tumor cell proliferation, metastatic dissemination, and heterogeneity. allows us to investigate 6PGD's function in the context of cancer. Glucose metabolism through this pathway primarily yields NADPH and ribose 5-phosphate, two building blocks for the synthesis of other components, as well as an antioxidant. The concentration of ROS in a cell is what determines its function. Unbalanced ROS contributes to cell death and cancer. The abbreviations used here are PPP (pentose phosphate pathway) and ROS (reactive oxygen species). Inhibitors of folate synthesis, such as sulfa drugs, are well-known as competitive p-aminobenzoate inhibitors (pABA). Since they mimic pABA so effectively, they could also attach to 2-amino-4-hydroxy-6-hydroxymethyl-7,8 dihydropteridine pyrophosphate to form the inhibitory compound sulfa-dihydropteroate (sulfa-DHP). This finding suggests that dihydropteroate synthase is only one of many processes that contribute to the development of resistance to sulfa medications. New therapies based on the DHP analogues might join the arsenal of antifolate treatments in the near future.

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