Inhibitory effects of some schiff bases and metal complexes on xanthine oxidase

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Abstract

Gout cancer, diabetes, and metabolic syndrome are all linked to xanthine oxidase deficiency. Schiff Bases and Metal Complexes, a bioactive substance, and its derivates were tested for their ability to inhibit the enzyme xanthine oxidase in the present study. The objective is to create bio-actives with antioxidant potential from Schiff Bases and Metal Complexes. Metal complexes, Schiff bases, hydrazines and natural acids were all employed to produce ester bonds using molecular docking techniques. The compounds' antioxidant and xanthine oxidase inhibitory activities were evaluated. 4-Metoksi S1 derivatives demonstrated a competitive inhibition of XO ability with IC50 value of 6,601 M and 4-Metoksi S1 was identified as the most active derivative. In the binding site of XO, the new 4-metoksi S1 derivatives bind to amino acid residues Leu257, Glu263, Thr262, Gly260, Ser347, Alkyl and Pi-Alkyl (Lys256, Ile353, Val259, Leu305, Ala346, Phe337). Testing for antioxidant capability of all compounds revealed that they were all very effective. Hybridization of Schiff Bases and Metal Complexes 4-metoksi S1 might lead to more potent xanthine oxidase inhibitors via molecular docking.

Keywords: schiff bases, metal complexes, xanthine oxidase, inhibitors

Introduction

Bioinorganic chemists have struggled to synthesize low molecular weight nickel (II) complexes that imitate superoxide dismutase (SOD) function, however certain complexes with strong catalytic activity have recently been discovered (Berry *et al.* 2004) [1]. Several Streptomyces species have been found to contain nickel-containing superoxide dismutase (Ni-SOD). Ni-SOD has an enzymatic activity of around 109 M1S1 per metal center, which is comparable to Cu-Zn SOD. Cancer cells have lower superoxide dismutase (SOD) activity than normal cells, according to Oberley and Buettner (Moriwaki *et al.* 1997) [8]. Because superoxide ion is hazardous to cells, nature must have devised a defense mechanism. At least one SOD is found in all organisms that utilize dioxygen and many that must survive in an oxygenated environment.

The superoxide radical is an unavoidable consequence of aerobic metabolism that, if left unchecked, can cause considerable cellular damage and has been linked to a variety of medical conditions. To avoid such negative repercussions, all oxygen-metabolizing organisms include superoxide dismutases, which are metallo-enzymes (SODs). The harmful radical is dis-proportionated to molecular oxygen and hydrogen peroxide by these SODs. The superoxide radical is an unavoidable consequence of aerobic metabolism that, if left unchecked, can cause considerable cellular damage and has been linked to a variety of medical conditions (Klinenberg *et al.* 1965) [6]. To avoid such negative repercussions, all oxygen-metabolizing organisms include superoxide dismutases, which are metallo-enzymes (SODs). The harmful radical is dis-proportionated to molecular oxygen and hydrogen peroxide by these SODs.

Zanthine oxidoreductase has two forms: XO (oxidation of xanthine) and ZD (dehydrogenation), which are both enzymes (XOR). Purines have a role in the last two stages of catabolism. Hypoxanthine oxidation, in particular, is catalyzed by these enzymes. Xanthine is converted to uric acid by the formation of uric acid from uric acid. XDH, on the other hand two electrons are transferred during the oxidation of the one molecule of nicotinamide as a substrate in each reaction step XO transports electrons from the adenine dinucleotide (NAD+) to the adenine dinucleotide (NAD+). hydrogen peroxide or superoxide are formed when oxygen molecules react with each otheranion.

The breakdown of DNA and RNA molecules produces purines, which accumulate both outside and internally in the human body. End product of purine catabolism, uric acid is excreted through the kidneys but also the digestive tract in humans and higher apes.There are two transporters in the kidneys that are capable of recycling uric acid: the URAT1 and the ABCG2 transporters, but only the ABCG2 transporters are able to do so in the colon. The enzyme uricase further oxidizes allantoin, a water-soluble compound, in most other species (Yu 2007) [12]. Aims of Study to assessment new xanthine oxidase inhibitor from some Schiff Bases and Metal Complexes constituents along with antioxidant potential. And Using molecular docking to explain changes for xanthine oxidase after effect Schiff base on enzyme.

Materıals and methods

An assay kit for the measurement of xanthine oxidase activity in a broad variety of samples is Ab102522.

Material

Milk, urine, serum, and plasma may also include tissue and cell culture extracts.

Equipments

Calibration Solutions for pH Meters with Glass Electrode and Glass Electrode Calibration Solutions (HCl, NaOH) A centrifuge is used to prepare the substrate. Using a Magnetic Hot Plate Stirrer for Buffer Preparation. Measurement and preparation of solid materials using analytical balancing. Absorbance may be measured using a microplate spectrometer.

Making and using different remedies

Hydrogen peroxide (H2O2) is used to generate color and fluorescence by breaking down the xanthine in the sample using an enzyme. Measurement of XO activity may be done with precision since color or fluorescence intensity has a direct correlation with XO content. The kit can detect xanthine oxidase concentrations of 1–100 mU in a reaction volume of 100 l.

Method

This product, previously known as the K710 Xanthine Oxidase Activity Colorimetric/Fluorometric Assay Kit, is made by BioVision, an Abcam subsidiary. This ab has the same 100-test size as ab102522. Healthy persons may have the enzyme xanthine oxidase (XO, EC 1.73.3.2) in their livers and intestines. Increased quantities of the hormone XO are released into the body as a result of certain liver diseases. This is because to the fact that blood XO levels may be used to identify liver disease, especially jaundice. Products containing European Authorization List (Annex XIV) compounds from Abcam will not be subject to REACH authorization under REACH regulations. Our clients are responsible for determining whether or not they require a REACH Authorization for their proposed use. Indicators for measuring physical activity are included in Table 1. Steps involved in performing an enzyme assay such as xanthine oxidase: Samples and standards should be placed in the wells. Reply into the mix. After around 10 to 20 minutes, analyze the samples using a microplate reader.

Table 1: Analyzing the activity of the enzyme Xanthine Oxidase

Stock solutions	Control cuvette	Sample cuvette
	Volume (µL)	Volume (µL)
500 mM fosfat	50	50
D.W	90	90
Enzyme sample	10	10
10 minutes incubation		
Substrate	-	60

Moleculer docking

In the study of drug-receptor interactions, a method known as molecular docking is often used. The outcomes of molecular docking are critical to a drug's capacity to attach to a receptor. The activity and affinity of drug candidates' molecules may be evaluated by evaluating their binding orientation to their chosen targets. to wit (Rahim *et al.* 2020) [11]. With the Avogadro chemical modeling program, the 3D structures of ligands were created (Hanwell *et al.* 2012) [4]. It was possible to minimize the amount of tiny molecules by using the MMF94x force field and the Avogadro's geometry optimization tool (Morris *et al.* 2009) [7]. Eight drugs were tested for potency and binding location using molecular docking software called Autodock. To determine the protein's coordinates, (Cao *et al.* 2014) [2], used PDB 3NVY (Cao *et al.* 2014) [2]. The quercetin molecule has been shown to have a two-dimensional structure at the binding location. There were two components added to the protein to produce the catalytic portion: molybdenum metal ions (Mo) and cofactor MTE (also known as Mn). Coordinate, grid box, and docking parameter files were generated using AutoDockTool All molecules received a Gasteiger partial charge. The grid mapping and docking algorithms were created using Autodock. Energy consumption A grid box with a 50 50 50 size was generated using the natural ligand center of the molecule in question. We were able to find every conceivable type of life using the Lamarckian genetic process. For each ligand, 1000 runs were performed to cover the whole conformational space using a population of 300, 10,000,000 million energy assessments, and a maximum of 27000 generations. According to AutoDock 4.2's binding energy, the 1000 docked sites were assessed on their ability to bind. For docking purposes, three of the eight bis-chalcone derivatives investigated were shown to be the most effective. The RMSD of docked conformers is used to organize them into clusters (RMSD). VMD was used to construct all of the molecular visualizations. The binding site was coated with quercetin and salicylic molecules in an early validation investigation. The crystal X-ray diffraction data was used to confirm the docking sites against the ligand's orientation and conformation. "bound" docking is a term used to describe this situation (Erickson *et al.* 2004). Allopurinol and oxypurinol, XO-inhibiting purines, were also employed as reference binding energies.

Results and discussion

Molecular docking

Experiments were conducted using the Schrödinger suite to understand the structure-activity relationship found in this work and forecast the possible interactions between produced chemicals and XO. The year is 2018 in New York City (Schrödinger Release 2018-2, New York City: Schrödinger, LLC).

The crystal structure of xanthine oxidase with PDB code 2E1Q was utilized for docking calculations.As shown in Table 2, the docking score and binding energy calculation were used to find the most promising variants. Important interactions including as hydrophobic regions, hydrogen bonding, polar contacts, and pi–pi bonds were visualized in the active pocket of xanthine oxidase using the Site map tool from the Schrodinger suite (Patel *et al.* 2010) [9]. The derivatives with higher docking scores than 4-metoksi S2 were retained for further synthetic operations, whereas the

others were rejected. Three-dimensional poses of two of the most 3-metoksi S2 and 4-metoksi S2 active molecules were seen and compared to 4-metoksi S1 and the standard medication Allopurinol**.** 3-metoksi S2-Cu, 3-metoksi S2-Pd, 4Metoksi S1-Cu were revealed as binding pocket residues implicated in the interaction. Docking studies of newly synthesized non-purine XO inhibitors revealed a comparable binding pocket (Li *et al.* 2013) [5]. Ommon binding interaction and in Table.3 metoksi S2 interaction details include amino acid and interaction with different bounds in the compound.

Table 2: Molecular docking studies and in vitro activities were compared

		IC50	Mol Dock Score	HBond
Unknown 1	3-metoksi S2	9,120	-155.568	-14.215
Unknown 1_1	3-metoksi S2-Cu	0,767	-250.616	-14.7373
Unknown 1_2	3-metoksi S2-Pd	6,601	-255.741	-11.9751
Unknown 1_3	4-metoksi S1	16,120	-162.186	-13.6719
Unknown 1_4	4Metoksi S1-Cu	0,942	-231.991	-6.72079
Unknown 1_5	4Metoksi S1-Pd	5,682	-255.401	-13.0533
Unknown 1_6	4-metoksi S2	6,601	-161.709	-11.0565
Unknown 1_7	4-metoksi S2-Cu	0,534	-276.306	-18.2608
Unknown 1_8	4Metoksi S2-Pd	5,458	-266.139	-13.8788

Table 3: Metoksi S2 interaction details

Interactions	Amino acids
Conventional Hydrogen Bond	Leu257, Glu263, Thr262, Gly260, Ser347
Alkyl and Pi-Alkyl	Lys256, Ile353, Val259, Leu305, Ala346, Phe337
Pi-Sigma	Leu257

Visual study of the 3D poses of metoksi S2 showed a tight arrangement of hydrophobic and polar residues surrounding the ligand that allowed for a confined channel in the binding cavity of the XO ligand. Figures 4.1, Figure 4.2, and Figure 4.3 show an interesting Pi-Sigma relationship between the hydrophobic residue PHE 798 of the active site and the Conventional Hydrogen Bond. The OH group of metoksi S2 and the polar residue, as well as the negatively charged Alkyl and Pi-Alkyl, showed a strong hydrogen bonding relationship. Figure.4 agreement with study study by (Shen and Ji 2009) [10].

It was discovered, however, that during imaging of metoksi S2, the hydrophobic residue MET 1038 was linked to the OH group of the phenyl ring. Figure 4.5 and Figure.6. Another hydrogen bond, comparable to 4-metoksi S1, was discovered between the OH group of metoksi S2 and the polar residue Figure. 7, Figure 4.8 and Figure 9.

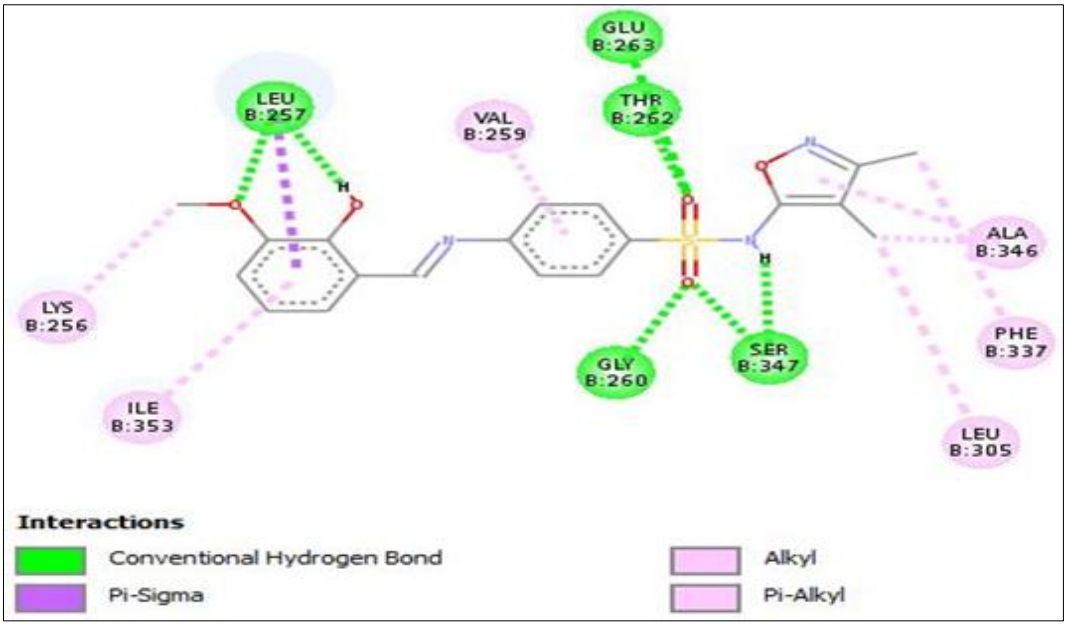

Fig 1: 3D pose for 4-metoksi S2

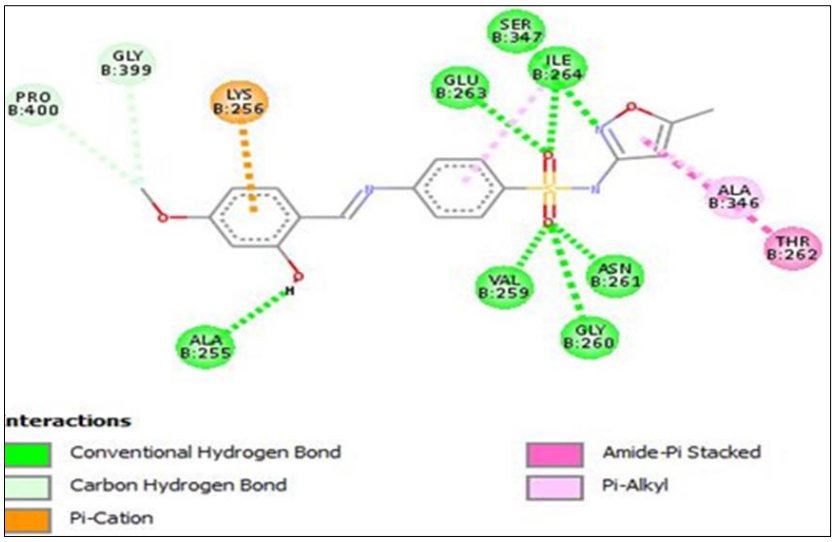

Fig 2: Interaction of Hydrogen Bond for 4-metoksi S2

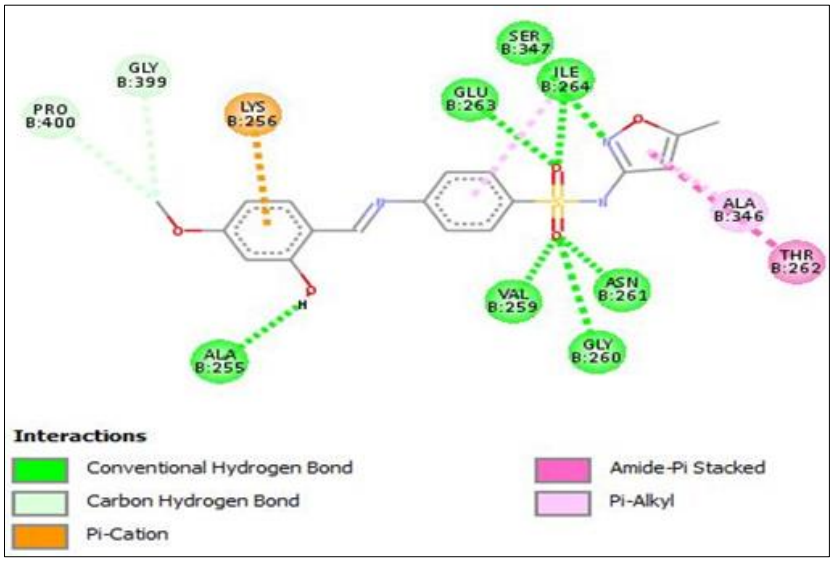

Fig 3: The 2Dinteraction map of 3-metoksi S2 with xanthine oxidase active site

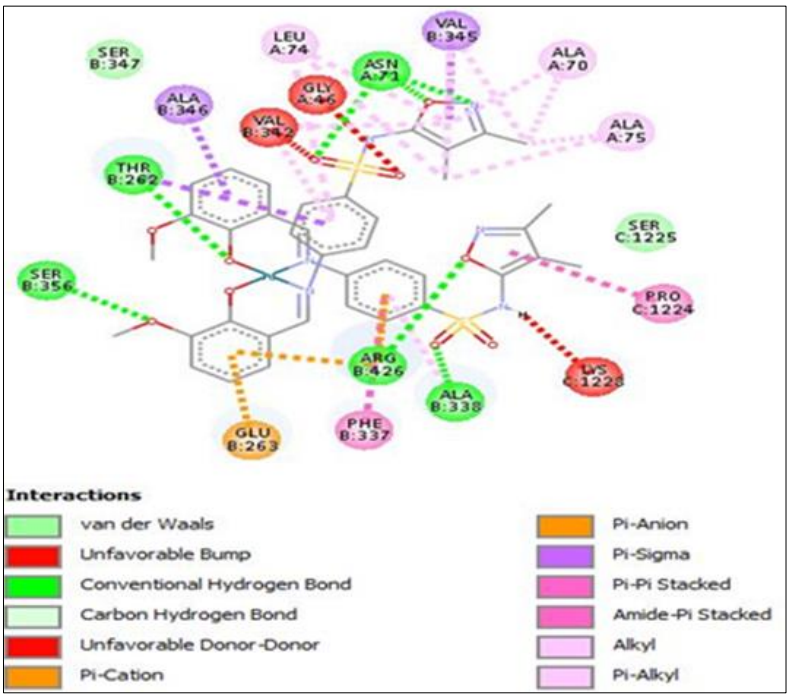

Fig 4: The 2Dinteraction map of 3-metoksi S2 with xanthine oxidase active site

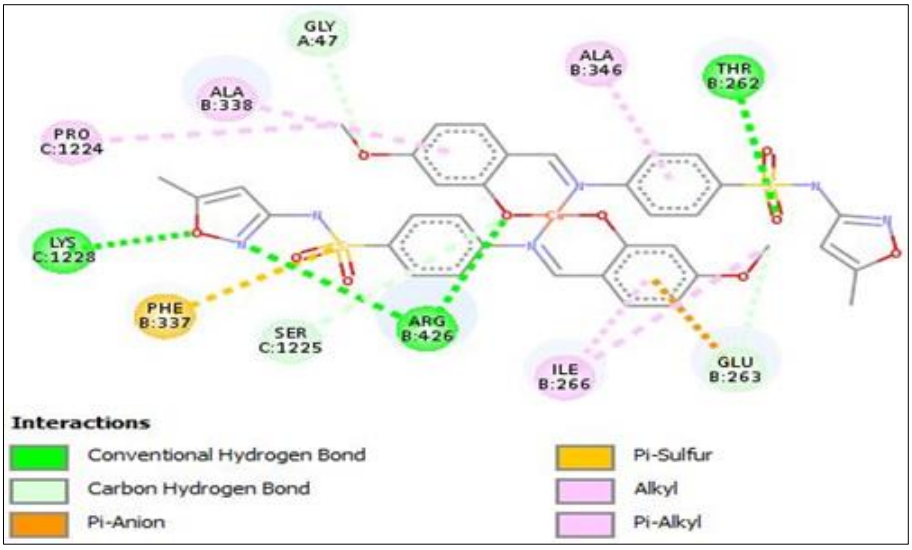

Fig 5: The 2Dinteraction map of 4-metoksi S2 with xanthine oxidase active site

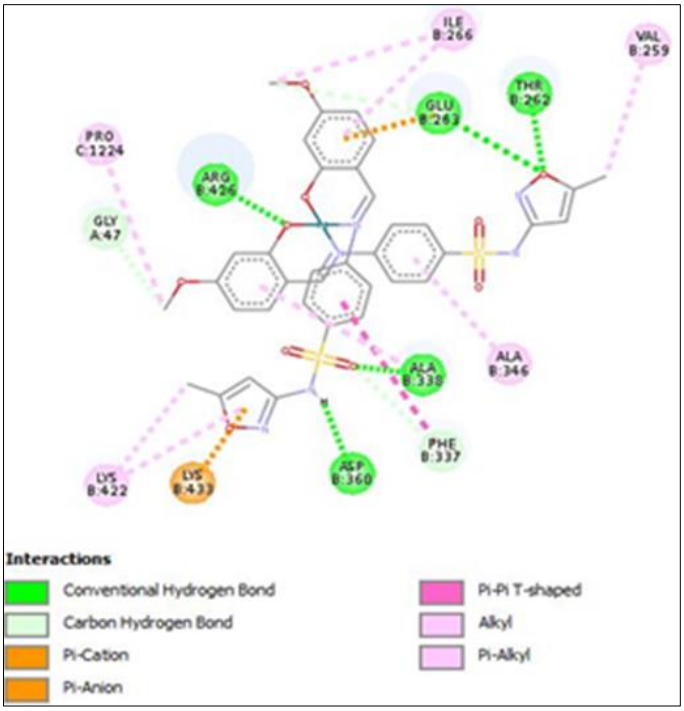

Fig 6: The 2Dinteraction map of **3**-metoksi S2 with xanthine oxidase active site

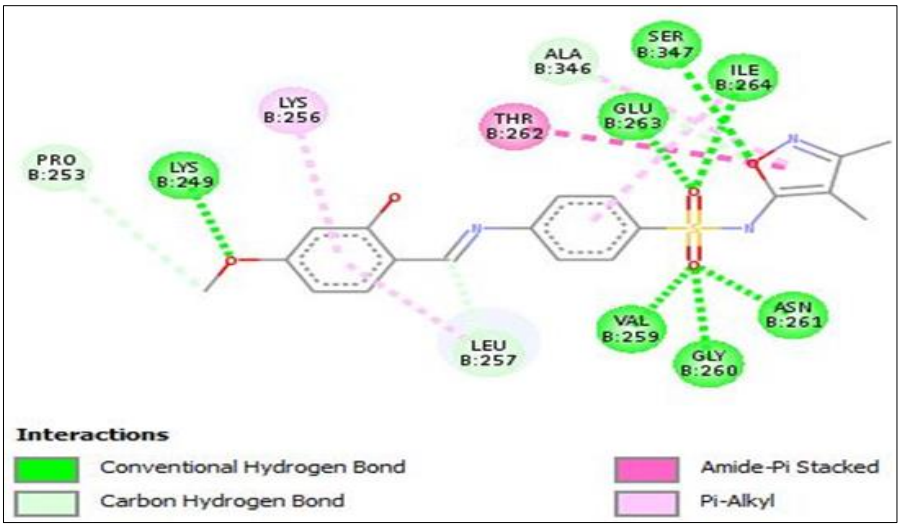

Fig 7: The 2Dinteraction map of 3-metoksi S2-Cu with xanthine oxidase active site

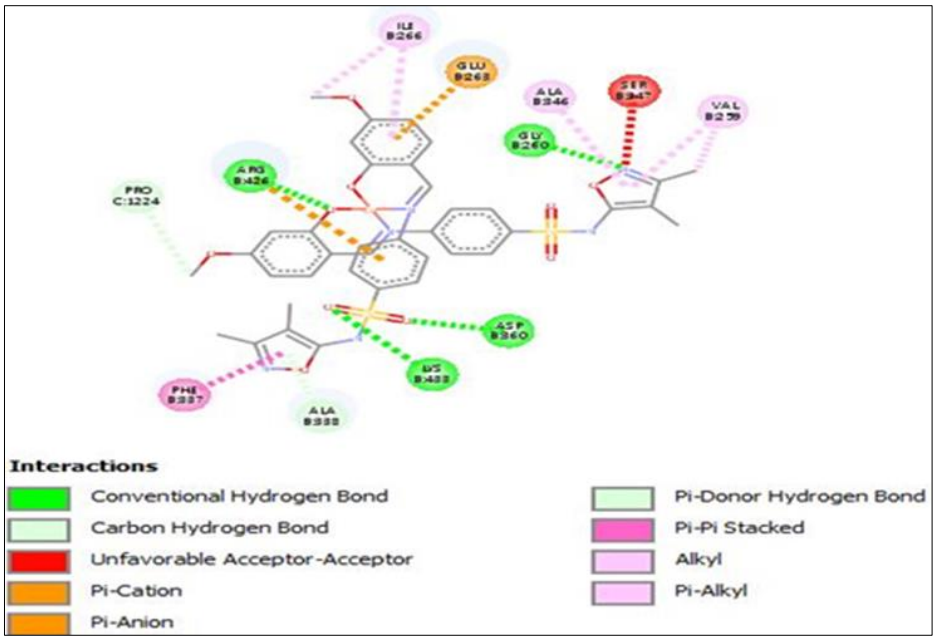

Fig 8: The 2Dinteraction map of 3-metoksi S2-Pd with xanthine oxidase active site

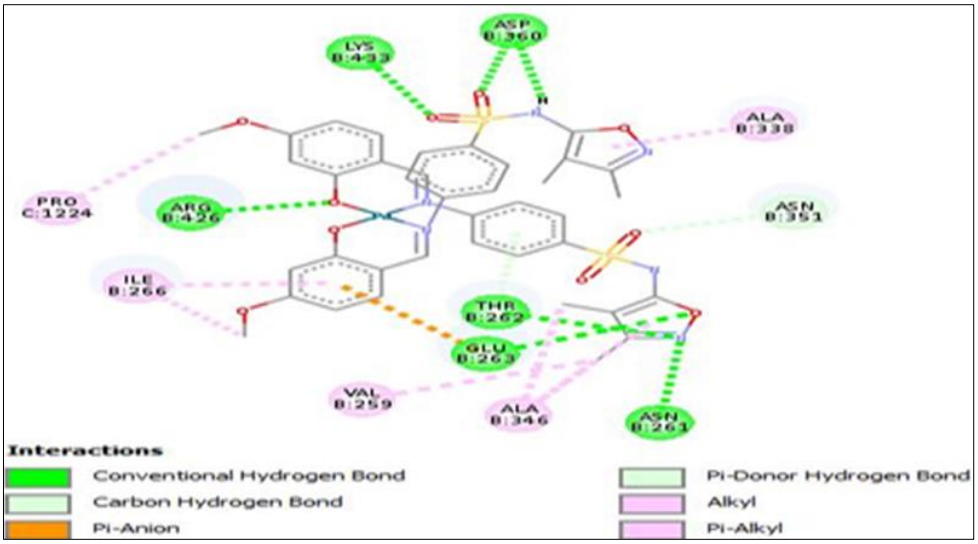

Fig 9: The 2Dinteraction map of 4Metoksi S2-Pd with xanthine oxidase active site

Xanthine oxidase plays a vital role in signaling in order to control the development and death of cells, which is why it is linked to cancer. In rapidly developing cancer cells, incorrect activation of Xanthine oxidase is linked to tumors and malignancies. If Xanthine oxidase is discovered to be a therapeutic target, current anticancer medications may be coupled with it to treat cancer resistance. Xanthine oxidase inhibitors in the form of chemical or molecular inhibitors have been widely used in research. The development of Xanthine oxidase inhibitors is based on metabolic ch Allopurinol was the sole XO inhibitor medicine on the market for more than 50 years. This enzyme has been associated to a variety of ailments, including diabetes, hypertension, and cardiovascular disease, therefore the search for novel XO inhibitors has accelerated in the recent decade. anges. There were two non-purine like inhibitors that have recently been authorized for the treatment of hyperuricemia, febuxostat and topiroxostat.

Since the discovery of allopurinol, various novel chemical structures have been discovered and investigated for their potential as XO inhibitors. When crystal structures are available, new inhibitor effects may be explained, and SAR models can be used to forecast the potency and selectivity of new medicines. It has been shown that the enzyme active site residues that provide the highest interactions with inhibitor compounds are Glu802, Arg880, Phe914, Phe1009 and Glu1261. It's possible that these chemicals' hydrogen bonding and aromatic–aromatic interactions explain the potency of the bulk of newly developed inhibitors.

More than one chemical structure has revealed how the presence of hydrogen bonding groups and nonpolar areas adjacent to one other is required for efficient inhibition of the enzyme, which is known to be XOactivity..'s Certain medications' great in vitro inhibition may not always translate to good in vivo inhibition, as has been the case with isocytosine derivatives. Many substances have been shown to interact with other residues inside the enzyme active site's narrow channel in addition to the five core residues. The

question of how to increase the frequency of enzyme-inhibitor interactions by taking into account amino acids around the active site entrance has now emerged.

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