Applied Chemistry Project

Project title Determination of effective *Plasmodium falciparum* dihydropteroate synthase inhibitors using molecular docking technique

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Academic year	2020		

Faculty of Science, Chulalongkorn University

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by
Miss Raveeporn Anantapattanapong
Miss Sirintra Kasemlonnapa

In Partial Fulfillment for the Degree of Bachelor of Science
Program in Applied Chemistry (International Program)
Department of Chemistry, Faculty of Science
Chulalongkorn University
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Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

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Date 25 December 2020

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Abstract

Nowadays, malaria is still a serious global health problem. The most severe form is caused by *Plasmodium falciparum* parasites. Dihydropteroate synthase enzyme (DHPS) plays an important role in genetics material synthesis of *P. falciparum* parasites. Nonetheless, an effective inhibitor of *P. falciparum* DHPS is currently not available due to drug resistance of the parasites. Therefore, the objective of the research is to determine the potent inhibitors of *P. falciparum* DHPS using molecular docking technique. Totally 19 putative inhibitors were docked to the DHPS wild-type and four mutants. Results showed high possibility that the sulfa inhibitors, SDX-DHP and STZ-DHP, are inactive with the mutant enzymes due to the absence of the interaction with residue PRO 438. Since the interaction with this amino acid could be found in the inhibitor PTA binding with any types of enzymes while the interaction with sulfa drugs only occurs in the wild-type class. According to our docking results, 7,8-dihydropteroic acid (ligand number 14) is the ligand with a fair potential to be active with the mutants as certain amino acid interactions similar to the inhibitor PTA were found. Moreover, ligand 14 might possibly be active with every enzyme due to the interaction with the key amino acid residues LYS 609 and ARG 686.

Keywords: Molecular docking/ Dihydropteroate synthase/DHPS/*Plasmodium falciparum*/Malaria/Computational chemistry method

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List of Abbreviation

Abbreviation	Explanation
ADT	AutoDockTools software version1.5.6
DHPS	Dihydropteroate synthase
DS viewer	Biovia Discovery Studio Visualizer Version 20.1
MT	Mutant
pABA	p-aminobenzoate
P. falciparum	*Plasmodium falciparum*
PTA	pteroate
RCSB-PDB	Research Collaboratory for Structural Bioinformatics–Protein Data Bank
RMSD	Root Mean Square Deviation
SDX-DHP	4-(((2-amino-4-oxo-3,4,7,8-tetrahydropterin-6-yl)methyl)amino)-N-(5,6-dimethoxy pyrimidin-4-yl)benzenesulfonamide
STZ-DHP	4-(((2-amino-4-oxo-3,4,7,8-tetrahydropterin-6-yl)methyl)amino)-N-(thiazol-2-yl)benzenesulfonamide
WHO	World Health Organization
WT	Wild-type

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CHAPTER 1

INTRODUCTION

1.1 Introduction to the research problem and significance

Malaria has killed one to three million people for several consecutive years, which is leading to significantly increase in the death rate in patients worldwide (Ibrahim et al., 2019). According to the report in 2016 from the World Health Organization (WHO), malaria was ranked the fifth killer for infectious disease that affects the mortality rate of billions of people over 91 countries in the world, especially in Africa, Asian, and Mediterranean regions. Also, WHO reported that the protozoan parasites in the Plasmodium family can cause the invasion of red blood cells in the host's body. There are five specific species of Plasmodium species that can transmit severe malaria to the human body, which consist of *Plasmodium knowlesi*, *Plasmodium Vivax*, *Plasmodium ovale*, *Plasmodium falciparum*, and *Plasmodium malariae*. (Tibon et al., 2020; Gupta et al., 2011). However, this study focuses on malaria infectious disease that is caused by the *Plasmodium falciparum* (*P. falciparum*) ,which is the most severe parasite of malaria developed in the gut of female mosquitoes in Anopheles genus (Lucantoni et al., 2013) The spread of the disease and transmission carry to the human body by the bites of the infected mosquitoes. Since over 80% of the major cases of malaria and about 90% of the high death rate worldwide of this severe disease come from *P. falciparum* species.(Gupta et al., 2011; Shibi et al.,2015). The most vulnerable group of people consist of young children, pregnant women, and migrant or traveler groups with lack of acquired immunity to *P. falciparum*. Furthermore, the most common symptoms from *P. falciparum* are headache, vomiting and nausea, fever, fatigue, diarrhea, chest and abdominal pain. Additionally, some debilitating clinical manifestations that are often associated with multi-organ dysfunction, which eventually leads to death such as liver failure, lung failure, and kidney failure generally occur in adults. According to WHO recommendations in 2012, pregnant women who live in the moderate to high risk region of malaria transmission should intermittently take sulfadoxine-pyrimethamine medicine as a preventive therapy, however, it is essential to follow a doctor's advices and recommendations for taking medicine of medical treatment safely during pregnancy.

In Thailand, *P. falciparum* is also a predominant species to naturally transmit malaria among human hosts by the infected female Anopheles. Although malaria is an infectious disease, this is crucial information to note that malaria can be transmitted from the infected person to another by blood. Thus, an organ transplantation, a blood donation, and sharing syringes could contaminate infected blood directly from patient to normal people. Besides, the endemic areas for Malaria in Thailand were mostly found only in hills and forests, especially in border areas. However, over the past five years, Thailand has made significant effort to

reduce the number of malaria cases within the countries. According to the report, the malaria-related morbidity has been continuously decreasing from 34,611 cases in 2014 to 5,425 cases in 2019, which drastically declined to 84% (Ministry of Public Health, 2019).

Also, Thailand has received financial support from external organizations since 2004, which is called the Global Fund to Fight AIDS, tuberculosis, and Malaria (GFATM). This is an important impact on the declining malaria cases. Thus, the integration at the same time amongst the improvement of economic status, funding support, and boost of the general health services system are the key successes of the malaria control in Thailand. Furthermore, Thailand has set an ambitious goal to eliminate malaria infectious disease by 2024 that aims to set zero in the local areas with malaria incidence (Bureau of Vector Borne Disease, Ministry of Public Health, 2019). Even though the significant reduction of malaria in Thailand came from those key strengths, one of the major challenges both Thai and worldwide face when uncontrolled malaria spread is the intensity drug resistance problem. The expansion of drug resistance in *P. falciparum* parasites in recent years increases significantly worldwide, which is the major problem to prevent and treat malaria (Tananchai et al., 2019). One of the effective ways to diminish this serious problem is to continuously develop novel antimalarial drugs.

Dihydropteroate synthase enzyme (DHPS) is a key enzyme involved in malaria infectious disease. The enzyme plays an important role in synthesis of genetic material in *P. falciparum*, which is related to nucleic acid biosynthesis of DNA and RNA of the parasites. Thus, DHPS is the attractive target of sulfa-based inhibitors such as sulfanilamides, sulfonamide, sulfone compounds, etc. The discovery of the clinically used sulfa drugs had been introduced since the 1940s, which is a sulfonamide. (Capasso and Supuran, 2014) Although the primary sulfonamides were discovered in vivo reduction of prontosil, which no longer exists, sulfa derivatives are continuously designed and developed in order to inhibit the enzyme as an antimicrobial drug (Clemente and Claudiu, 2014). There are some reports of *P. falciparum* resistance strains to sulfa drugs (Khalil et al., 2003). Therefore, it is necessary to investigate the reason for this drug resistance as well as to find new compounds that are active against these resistance strains.

In this research project, we hypothesized that the drug resistance may come from different ligand-enzyme interactions. Therefore, molecular docking techniques were used to investigate such interactions. Toward this goal, five crystal structures DHPS of *P. falciparum*, one wildtype (WT) and four mutations, were selected. In order to increase a chance to get new active compounds, non-sulfa compounds were considered for molecular docking investigations. Hopefully, this research project would be useful for the further studies of antimalarial drug development and design in order to decrease the rate of drug resistance and the death rate of the patients who suffer from malaria infectious disease.

1.1.2 Dihydropteroate synthase

DHPS is a crucial enzyme in the folate biosynthesis pathway, which is involved in metabolism and genetic materials synthesis of *P. falciparum* malaria parasites (Hevener et al., ,2010) Since the pathway of folic acid synthesis is absent in higher classes of eukaryotes, the pathway is not found in plants, animals, and humans (Dalia et al., 2014). According to the literature search, one of the important classes of DHPS inhibitors is the sulfonamide group which is usually sulfanilamide that acts as pABA substrate analogues because sulfanilamide group are the competitive inhibitors of DHPS (Chotpatiwetchkul et al., 2017). Since the sequencing of DHPS genes are totally different in amino acid, its problem has affected the mechanism of drug resistance (Triglia et al., 1997). However, development of new sulfa-drug agents against this enzyme is needed because DHPS has an increasing resistance rate from mutations in the enzyme (Babaoglu et al., 2004; Chotpatiwetchkul et al., 2017).

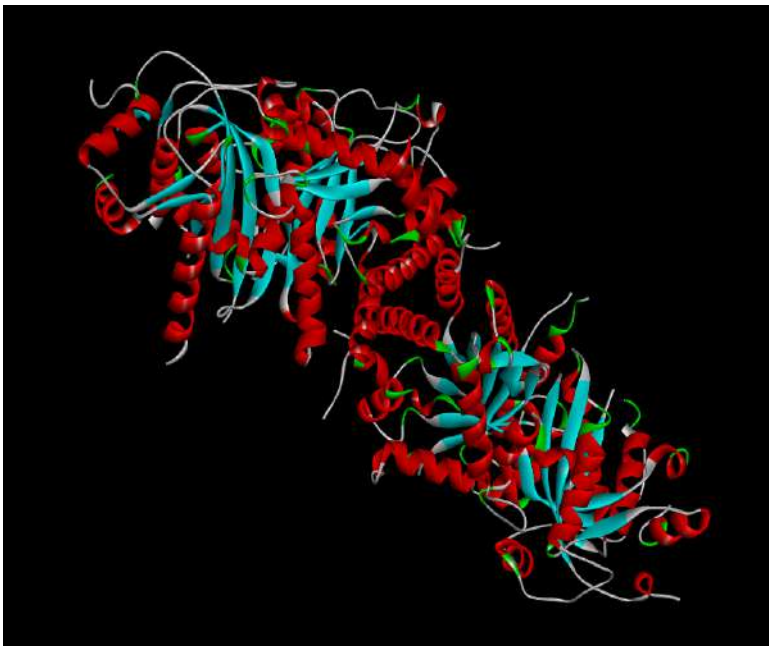


Figure 1.1 3D structure of dihydropteroate synthase enzyme

1.1.3 DHPS inhibitors

DHPS enzyme acts as the biocatalyst in combining pteridine with para-amino benzoic acid (pABA) to form dihydropteroate. (Hammoudeh et al., 2014). This compound will be further used for a synthesis of folates, which are key chemical substances in a nucleotide biosynthesis pathway of bacteria and protozoa. As sulfa drugs family such as sulfadoxine (Figure 1.2) have the analogue structure to pABA, they compete with pABA at the DHPS active site (competitive inhibitors, Figure 1.3), forming sulfa drug–pterin adducts instead of dihydropteroate. Therefore, they can inhibit the synthesis of DNA and RNA nucleotides of bacteria and protozoa and thus are used to treat a broad range of bacterial and protozoal infections.

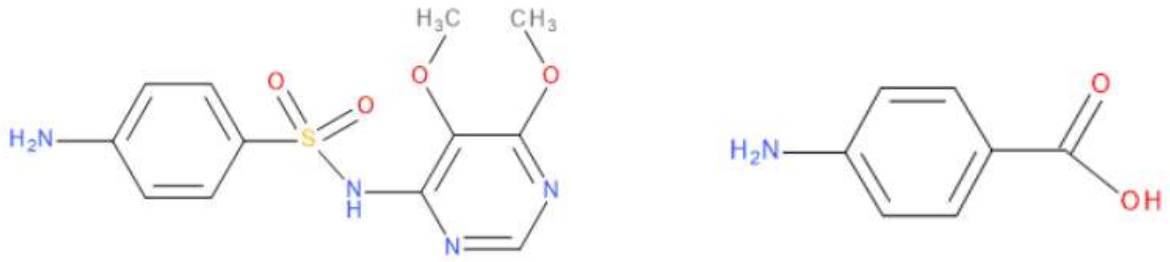


Figure 1.2 Sulfadoxine acts as the competitive inhibitor of pABA

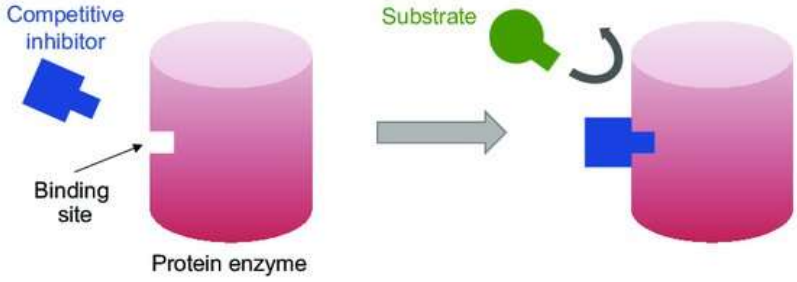


Figure 1.3 The principle of competitive inhibitor (Cichonska A., 2018, p.27)

1.2 Research Objectives

1.2.1 To study ligand-enzyme interactions of DHPS enzyme of *P. falciparum* in malaria for both wildtype and mutants using molecular docking technique.

1.2.2 To determine an effective inhibitor for DHPS.

1.3 Literature search

Chitnumsub et al. (2020) tried to find a reason for the increasing rate of resistance in *P. falciparum* parasites, which is one of the major obstacles in antimalarial-drug discovery. They focused on a bifunctional enzyme, which is 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) and dihydropteroate synthase (DHPS) enzyme. Thirteen structures of the enzyme both wild-type (WT) *P. falciparum* and sulfa-resistant mutants, which included both apoenzyme and the complexes with pteroate (PTA) and sulfa compounds, were solved in order to understand the mechanism of resistance and design a novel antimalarial drug. They found that PTA, which is an active inhibitor for both the WT and mutants without steric constraint at the active site. However, sulfa inhibitors stay outside of the substrate envelope, which is surrounding resistance mutations. The decline in the binding affinity of the compound to mutant residue occurred from the steric conflict between both of them, which can boost the flexibility of loop D2 in mutants. Therefore, the mutants are more increased in the activity of enzymes than WT from the results from kinetic data.

Based on the results, DHPS enzymes in *P. falciparum* parasites (PfDHPS) structural insights are the main problems for novel antimalarial drug development because substrate envelope–compliant DHPS inhibitors are less vulnerable to resistance mutations.

Stratton et al. (2015) studied the alternative inhibitor targeting in DHPS, which may be effective for the treatment of bacterial infectious diseases such as the disease caused by Staphylococcus aureus (*S. aureus*). The catalytic mechanism of DHPS generates the formation of dihydropteroate (DHP) through the condensation of pABA with 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPP). Therefore, the substrate binding sites of para-aminobenzoic acid (pABA) on dihydropteroate synthase enzymes in *P. falciparum* and *S. aureus* were calculated from equilibrium binding isotope effects and density functional theory (DFT). From the literature, DHPS in bacteria, which is Staphylococcus aureus (S. aureus), also called SaDHPS in short, is the monofunctional enzyme while DHPS from *P. falciparum* (PfDHPS) is a bifunctional enzyme with HPPK being as a PfHPPK as above-mentioned. However, the crystallographic structure of PfHPPK−DHPS is unavailable for study that may impact on lacking the useful and relevant information of both PfHKK and PfDHPS such as protein-substrate interactions. Therefore, binding isotope effects (BIEs) and computational modeling were executed to examine the information about the differences between the pABA binding sites of SaDHPS and PfHPPK−DHPS. The result from BIEs was reported that pABA binding environments of SaDHPS are less sterically constrained vibrational environments.

Garg et al. (2009) studied the synergy of sulfadoxine and pyrimethamine in order to inhibit the DHPS enzyme and dihydrofolate reductase (DHFR) respectively. Also, the combination of these two has an efficiency to decline the emergence of drug resistance by blocking DNA biosynthesis of *P. falciparum*. Bikaner is known for one of the cities in northwest region of India, where are found the severe and uncomplicated cases of sulfadoxine and pyrimethamine resistance of *P. falciparum*, which are related to the point mutation of DHPS and DHFR respectively. Few isolates of DHPS mutation were observed only at point A437G. By contrast, the predominance of isolates expressed the double mutant allele for DHFR. Furthermore, S587F, N666K and C668W in the PfDHPS are novel mutations that point mutations at 666 and 668 codons were likely to see a bend formation in the big loop part of the DHPS enzyme. Also, it would impact on the binding of the drug to the enzyme. In this study, molecular docking between sulfadoxine and this mutated structure was used to identify the reduction of binding affinity to the enzyme.

Mccullough and Maren (1973) studied the effective inhibitor of DHPS and its activity in *E. coli*, which is various of diphenylsulfones and sulfonamides. For example, 4, 4'-Diaminodiphenylsulfone (DSS) has an activity, or I_{50} equal to 2×10^{-5} M that is one of diphenylsulfones group used as both antibacterial and antimalarial drugs. However, pABA plays an important role in the reversal of this activity of DSS, which is like those of the sulfonamides. The results of several activities of both sulfones and sulfonamides against

DHPS enzymes showed that I_{50} values of sulfadiazine, sulfanilic acid, and sulfanilamide are equal to 1.1 x 10^{-5}, 3.5 x 10^{-5}, and 3.2 x 10^{-4} respectively. A comparison of the inhibitory properties between sulfones and sulfonamides on DHPS from I_{50} values could be concluded that DSS was the most potent inhibitor in the sulfones group while sulfadiazine was the most effective inhibitor in the sulfonamides group. In conclusion, DSS, which is the most active of the sulfone group, is half active in sulfadiazine.

Kasekarn et al. (2004) studied the hydroxymethyldihydropterin pyrophosphokinase and dihydropteroate syntheses of *P. falciparum* (pfPPPK-DHPS) that acted as the bifunctional enzyme. In order to express a DHPS-deficient in *E. coli*, it was notably controlled under the T5 promoter. In this study, the processes were performed at the optimal temperature during 37 to 45 °C and pH 10 with appropriate concentration of sodium chloride at 0.2 M and potassium chloride at 0.4 M. that can activate the activity of enzyme. According to the articles, 50 % of the enzyme activity was inhibited by urea, which is the concentration at 0.9M due to the fact that the higher salts concentration is an inhibitor. Besides, the investigation of the recombinant pfPPPK-DHPS using kinetic properties showed that sulfathiazole was an effective inhibitor of pfPPPK-DHPS. By constant, sulfanilamide was less inhibitory.

CHAPTER 2

COMPUTATIONAL DETAIL

2.1 List of computational software

- HyperChem version 8.0.10
- BIOVIA Discovery Studio Visualizer version 2020
- AutoDockTools version 1.5.4
- VEGA ZZ version 3.2.1

2.2 List of receptors, inhibitor, and ligands data

2.2.1 List of receptors

In order to investigate the reason for drug resistance in DHPS, the enzyme-ligand interactions and the key binding residues in both WT and mutants must be identified. Totally, eight experimentally determined 3-dimensional structures of DHPS in complexes with different inhibitors were obtained from the protein data bank (PDB).

Table 2.1 Each type of enzyme-inhibitor DHPS.

PDB ID	Enzyme	Inhibitor
6JWU	WT	STZ-DHP
6JWR	WT	PTA
6JWV	single mutation at A437G	STZ-DHP
6JWS	single mutation at A437G	PTA
6JWW	triple mutations at S436F/A437G/A613T	STZ-DHP
6JWT	triple mutations at S436F/A437G/A613T	PTA
6JWZ	triple mutations at S436F/A437G/A613S	SDX-DHP
6KCL	double mutations at A437G/K540E	Pterin/pHBA

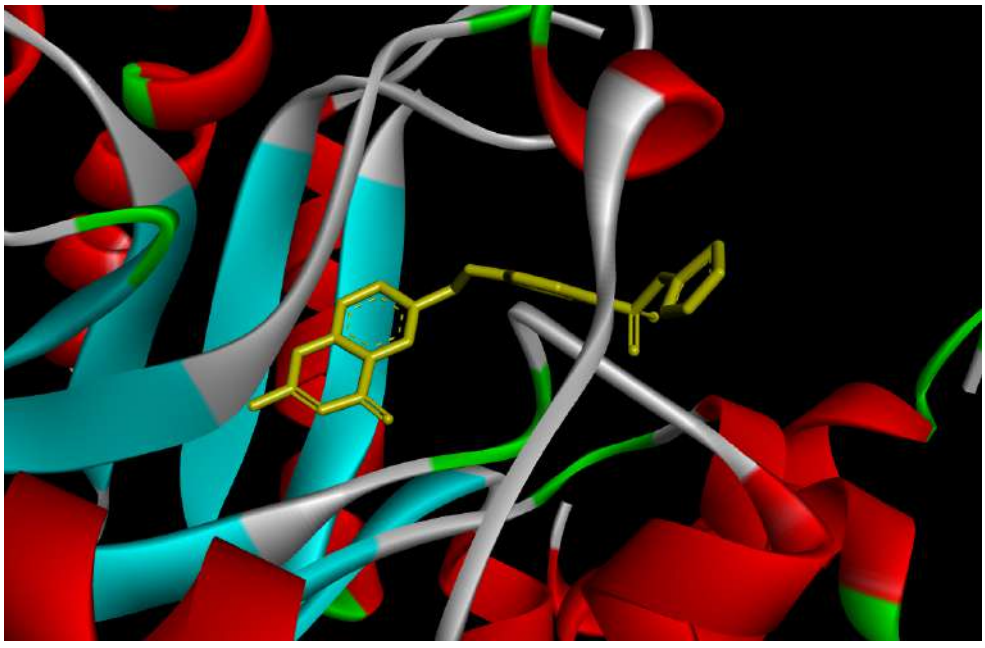


Figure 2.1 X-ray structure of WT DHPS in complex with STZ-DHP (PDB ID 6JWU)

2.2.2 List of inhibitors

- Pteroate (PTA)
- SDX-DHP
- STZ- DHP

The X-ray crystallography structure of all three inhibitors was downloaded from PDB. PTA is active in both WT and mutants while SDX and STZ are active only in WT, therefore these three compounds were used in the analysis in order to investigate the key binding residues.

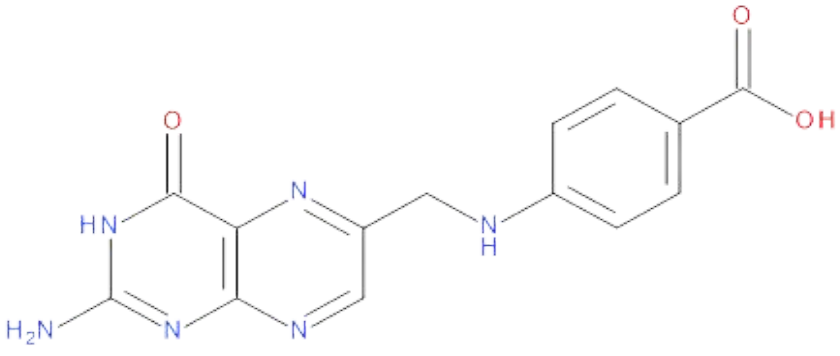


Figure 2.2 The chemical structure of PTA

2.2.3 List of ligands

To identify potential targets of DHPS, totally nineteen ligands were collected from literature (Chaparro et al., 2001; Clemente and Claudiu, 2014; Dalia et al., 2014; Kasekarn et al., 2004; Krudsood et al., 2005; Lawrence et al., 2005; Lumb et al., 2009; Mccullough and Maren, 1973; Nzila, 2006; Rébeillé et al., 1997; Wang et al., 1997; Zhao et al., 2012). Also, the two-dimensional structures of all ligands were built using Biovia Draw software, in which ten ligands are sulfa groups (Figure 2.3) and nine ligands are non-sulfa groups (Figure 2.4). However, this is important to note that a number in the parenthesis symbol of each ligand denoted as (number) and followed by the name of the ligand, for example, (3) sulfanilamide means that ligand number three is sulfanilamide.

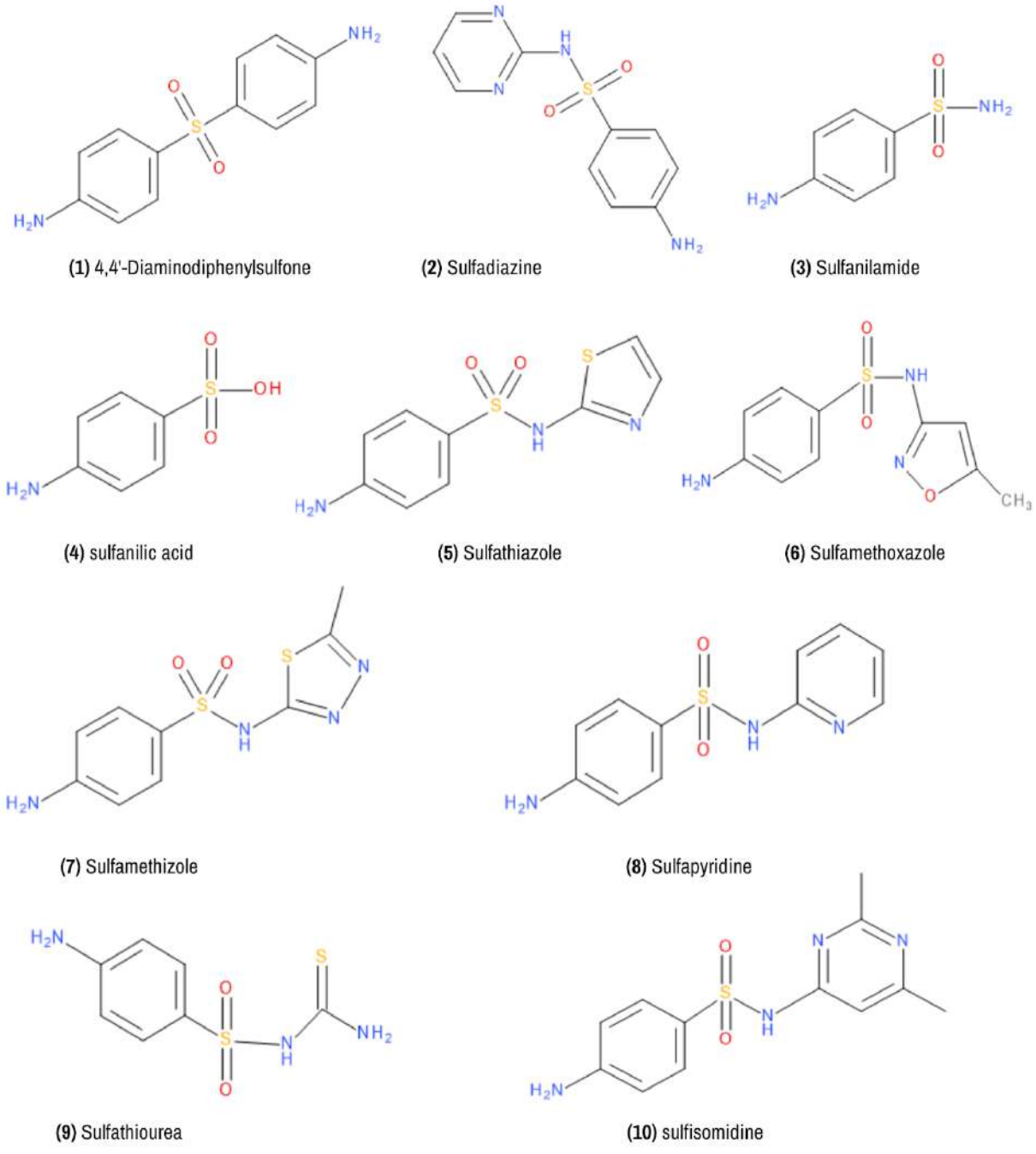
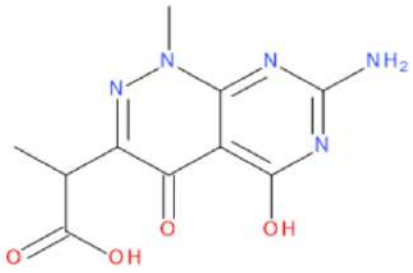
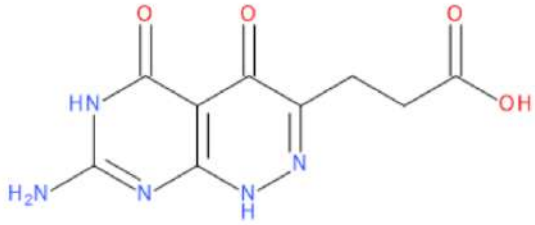


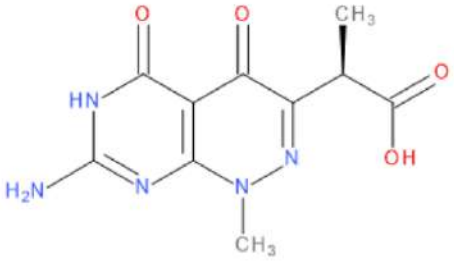
Figure 2.3 Chemical structures of sulfa-group ligands.



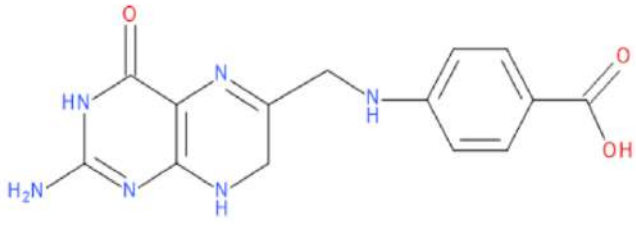
(11) 2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid



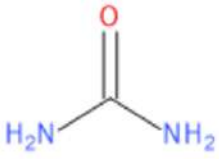
(12) 3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid



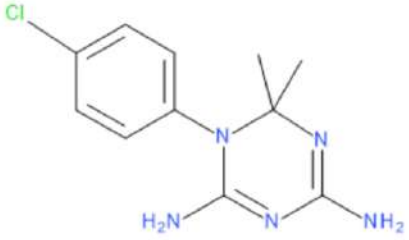
(13) 7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6H-dione



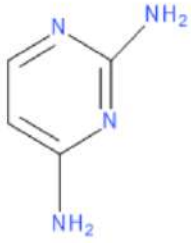
(14) 7,8-Dihydropteroic acid



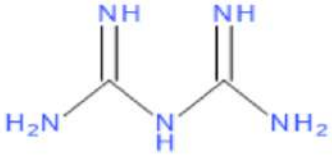
(15) Urea



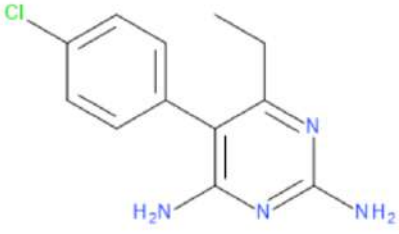
(16) Cycloguanil



(17) 2,4-Diaminopyrimidine



(18) Biguanide



(19) Pyrimethamine

Figure 2.4 Chemical structures of non-sulfa group ligands.

2.3) Experimental procedure

The concise explanation of procedure below has shown an example of 6JWU receptor and ligand number 1 from topic 2.2.3 Therefore, the others were performed as the same steps but in alternative substances.

2.3.1 Preparation of receptor structure

First of all, the X-ray crystallography structure of DHPS enzyme was retrieved from the Protein Data Bank (PDB ID: 6JWU). The DS viewer was used for the preparation of a 6JWU receptor. Only chain A of the enzyme was chosen to conduct molecular docking. Ligands and water molecules were removed. Then, a 6JWU structure with only chain A from the first step was put in the ADT. Polar hydrogens were added and the Gasteiger atomic charges were assigned.

2.3.2 Preparation of inhibitor structure

The 6JWU structure was opened with the DS viewer. Then, the inhibitor in Chain A was kept while all the other molecules were removed.

2.3.3 Preparation of ligand structure

The three-dimensional structure of each ligand was built using the HyperChem. Then, geometry optimization using the molecular mechanics method with MM+ force fields was carried out. After that, the optimized structure was opened in the ADT. Non-polar hydrogens were merged. Rotatable bonds were defined for the flexible docking. All necessary parameters were added.

2.3.4 Molecular docking calculation

For molecular docking calculation, AutoDock Vina and VEGA ZZ were used. The grid center was set using the coordinate of center of mass of inhibitor structure in the 6JWU. The grid box of size 30 x 30 x 30 $Å^3$ was used.

2.3.5 Analysis of enzyme-ligand interactions

In the docking calculation output file, there are nine docked configurations, and they are sorted according to their binding affinity. The first rank structure, a structure with the highest binding affinity, was selected. Then, the DS viewer was used to investigate the interaction between receptor and ligand in both 3D structure and 2D diagram.

Chapter 3

RESULTS AND DISCUSSION

3.1 Comparison of binding modes in WT and mutants DHPS

In order to investigate the reason for drug resistance of DHPS, the docked configurations of each ligand to four mutants (6JWV, 6JWW, 6JWZ, and 6KCL) were compared with that to WT (6JWU). Docked configuration of each ligand to enzyme will be denoted as ligand number_PDB code of enzyme, for example, 3_6JWV means docked configuration between ligand 3 and 6JWV. For each ligand, RMSD (root-mean square deviation) between each docked configuration to mutants and that to WT was calculated. Two docked configurations with RMSD value of less than 2.00 Å are considered as having similar binding mode. From the comparison of binding patterns, the results can be divided into four groups.

3.1.1 Ligands with binding modes in mutants different from that in WT

Ligands in this group (Group 1) bind with each mutant differently from that with WT. RMSD values compared to the WT are higher than 2.00 Å. However, docked configurations to some mutants are similar to each other in some ligands (subgroup 1A) while they are all different in some ligands (subgroup 1B).

3.1.1.1 Subgroup 1A

In this subgroup, ligands with docked configurations to some mutants are similar to each other. There are 9 compounds in this subgroup.

Ligand 3

Table 3.1 RMSD value of ligand 3

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	13.61	0			
6JWW	2.89	11.91	0		
6JWZ	5.83	11.98	5.08	0	
6KCL	2.65	13.13	2.06	5.29	0

According to the RMSD values, 3_6KCL has the closest binding position with 3_WT when comparing with other ligands, with the RMSD value of 2.65 Å, which is the smallest value amongst others. In addition, 3_6JWV has the greatest dissimilarity in binding position amongst others in the report of RMSD value 13.61 Å. Remarkably, only a pair amongst ligands binding with mutants was found to have similar binding site, which could refer to the same activity between the two enzymes. The RMSD value between 3_6JWW and 3_6KCL is 2.06 Å, in which the value marginally surpasses the standard value 2.00 Å. Apart from the pair, binding sites of all ligand binding with mutant receptor configurations are clearly differing with one another.

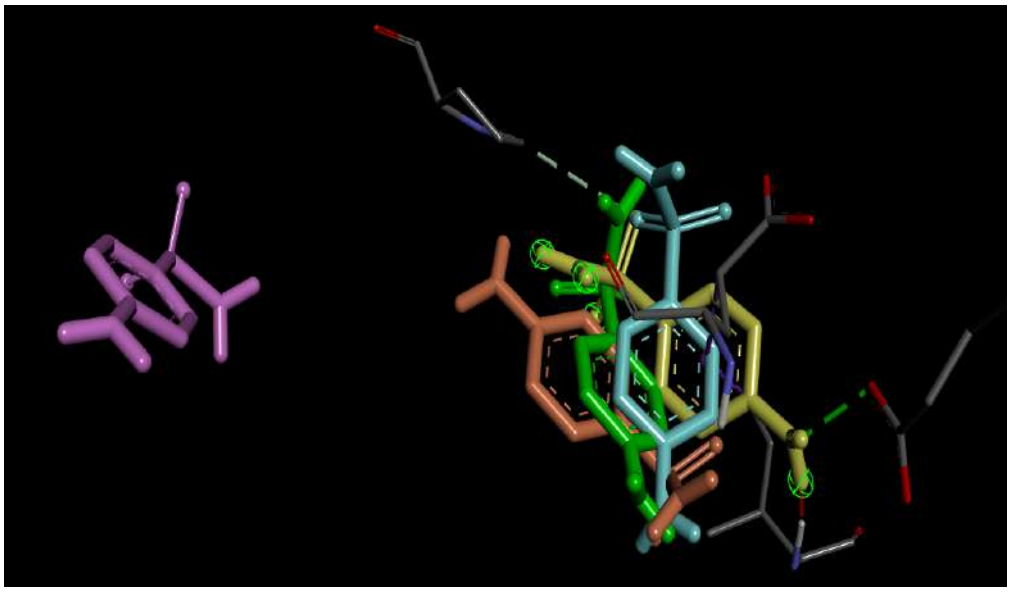


Figure 3.1 Comparing the similarity amongst Ligand 3 binding to different type of enzymes

Ligand 4

Table 3.2 RMSD value of ligand 4

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.93	0			
6JWW	2.20	6.40	0		
6JWZ	6.23	1.92	6.44	0	
6KCL	10.55	9.44	11.72	9.527	0

According to the result, 4_6JWW has the closest binding position with 4_WT, due to the RMSD value 2.20 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 4_6KCL, with the RMSD value of 10.55 Å. From the observation, 6JWV_Ligand number 4 and 6JWZ_Ligand number 4 are very much alike in terms of the binding site as the result of the RMSD value, 1.92 Å, hence a similar interaction and activity.

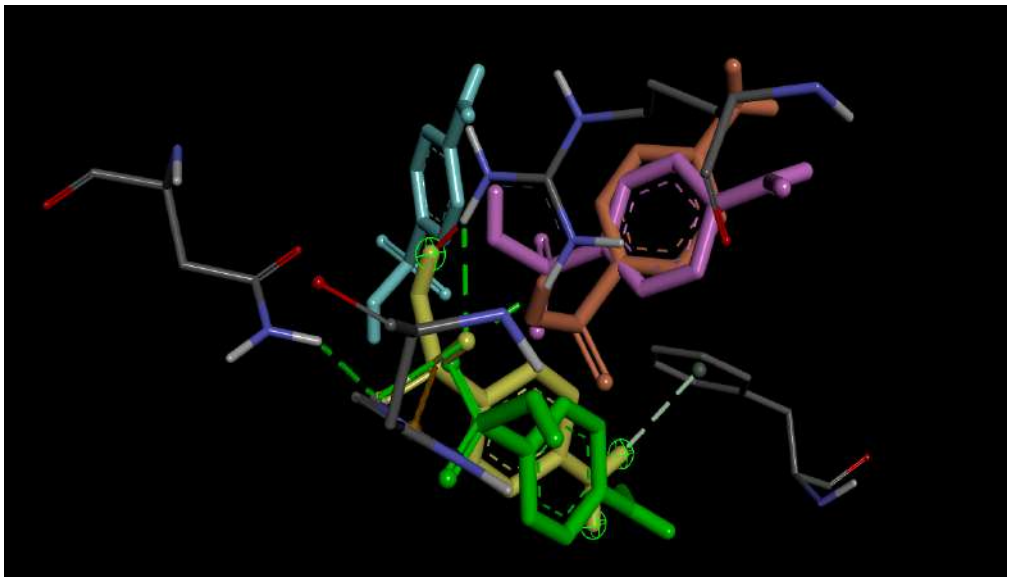


Figure 3.2 Comparing the similarity amongst Ligand 4 binding to different type of enzymes

Ligand 7

Table 3.3 RMSD value of ligand 7

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.94	0			
6JWW	11.15	8.99	0		
6JWZ	6.24	0.68	8.79	0	
6KCL	5.67	0.61	9.26	0.92	0

According to the result, 7_6KCL has the closest binding position with 7_WT, due to the RMSD value 5.67 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 7_6JWW, with the RMSD value 11.15 Å.

Remarkably, 7_6JWV, 7_6JWZ, and 7_6KCL are very much alike in terms of the binding site as the consequence of the RMSD value. The RMSD value of these pairings- 7_6JWV and 7_6JWZ, 7_ 6JWV and 7_6KCL, and 7_6JWZ and 7_6KCLare 0.68 Å, 0.61 Å, 0.92 Å, respectively. Therefore, these three ligands binding with mutant receptors consequently have a highly similar binding site with one another.

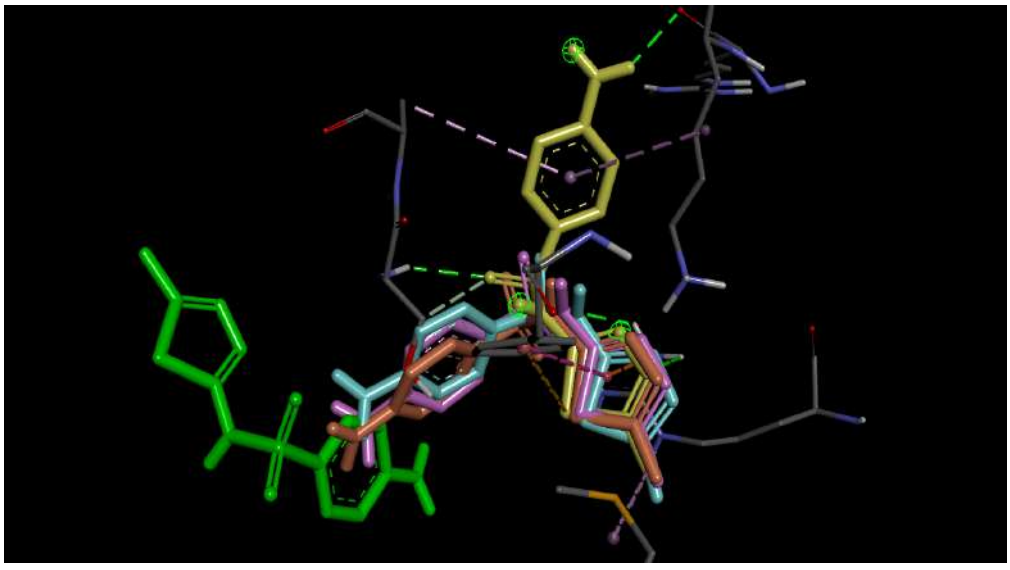


Figure 3.3 Comparing the similarity amongst Ligand 7 binding to different type of enzymes

Ligand 10

Table 3.4 RMSD value of ligand 10

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	4.78	0			
6JWW	2.62	4.43	0		
6JWZ	4.79	0.51	4.46	0	
6KCL	8.98	9.06	8.36	8.95	0

According to the result, 10_6JWW has the closest binding position with WT, due to the RMSD value 2.62 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 10_6KCL, with the RMSD value of 8.98 Å. From the observation, 10_6JWV and 10_6JWZ are very much alike in terms of the binding site as the result of the RMSD value, 0.51 Å, hence a similar interaction and activity.

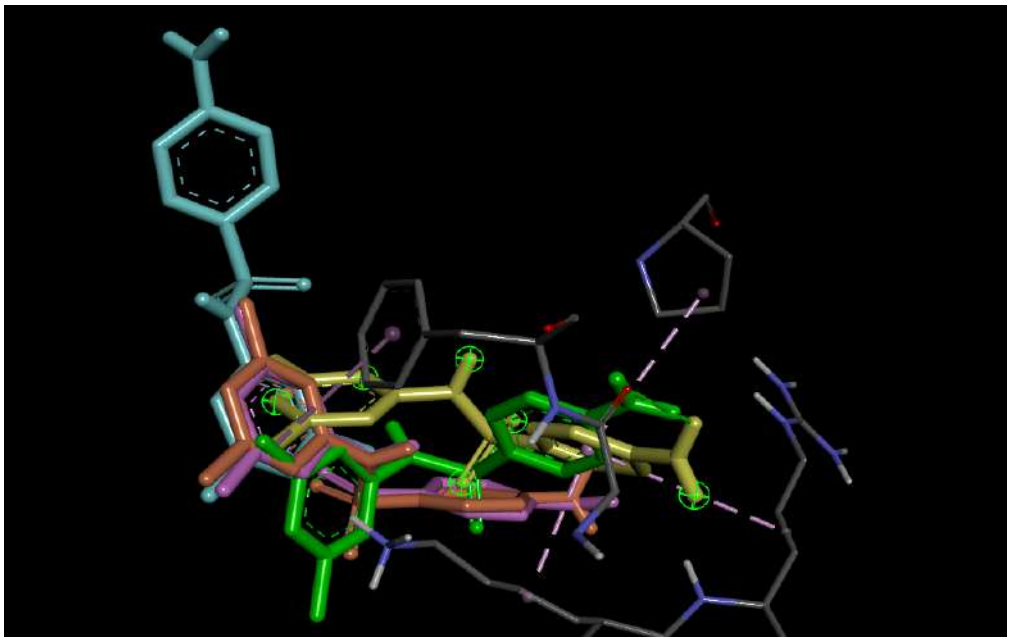


Figure 3.4 Comparing the similarity amongst Ligand 10 binding to different type of enzymes

Ligand 11

Table 3.5 RMSD value of ligand 11

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.53	0			
6JWW	7.71	5.57	0		
6JWZ	5.64	0.81	5.55	0	
6KCL	5.17	6.16	7.54	6.45	0

According to the result, 11_6KCL has the closest binding site with WT, due to the RMSD value 5.17 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 11_6JWW, with the RMSD value 7.71 Å. In addition, 11_6JWV has a proximate similarity to 11_6JWZ in respect of the binding position, according to a report of the RMSD value, 0.81 Å.

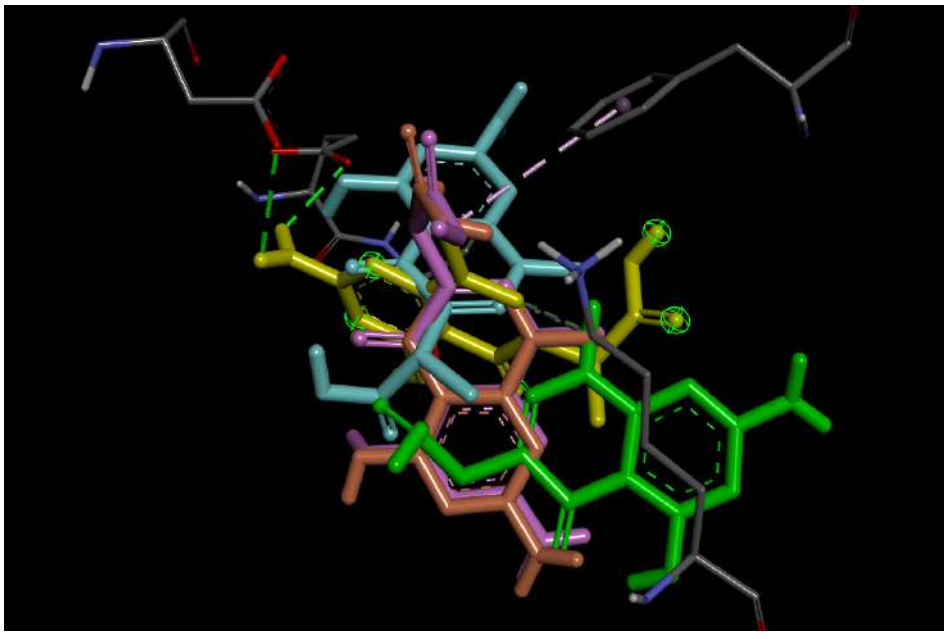


Figure 3.5 Comparing the similarity amongst Ligand 11 binding to different type of enzymes

Ligand 12

Table 3.6 RMSD value of ligand 12

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	6.89	0			
6JWW	8.51	8.71	0		
6JWZ	6.75	1.27	8.67	0	
6KCL	7.79	7.73	10.55	8.24	0

According to the result, the closest mutant binding with WT is 12_6JWZ due to the RMSD value 6.75 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 12_6JWW, with the RMSD value 8.51 Å. In addition, 12_6JWV has a proximate similarity to 12_6JWZ in respect of the binding position, according to a report of the RMSD value, 1.27 Å.

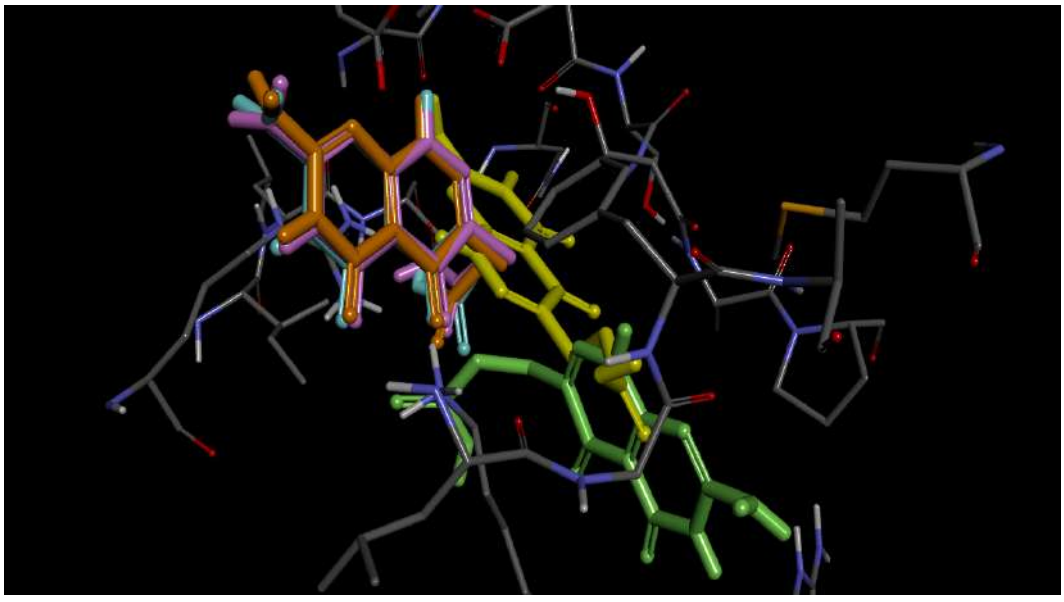


Figure 3.6 Comparing the similarity amongst Ligand 12 binding to different type of enzymes

Ligand 14

Table 3.7 RMSD value of ligand 14

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.39	0			
6JWW	9.95	10.09	0		
6JWZ	5.67	0.75	10.24	0	
6KCL	5.58	0.73	10.31	0.64	0

According to the result, 14_6JWV has the closest binding position with 14_6JWU (WT), due to the RMSD value 5.39 Å. Nonetheless, the variation of the RMSD value amongst 14_6JWV, 14_6JWZ, and 14_6KCL are highly insignificant, compared with the WT. As a report of the data, those three aforementioned configurations- 14_6JWV and 14_6JWZ, 14_6JWV and 14_6KCL, and 14_6JWZ and 14_6KCL - collaboratively have the RMSD value 0.75 Å, 0.73 Å, 0.64 Å, respectively. They are extremely identical in aspect of the binding site, hence the interaction and the activity. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 14_6JWW, with the RMSD value of 9.95 Å.

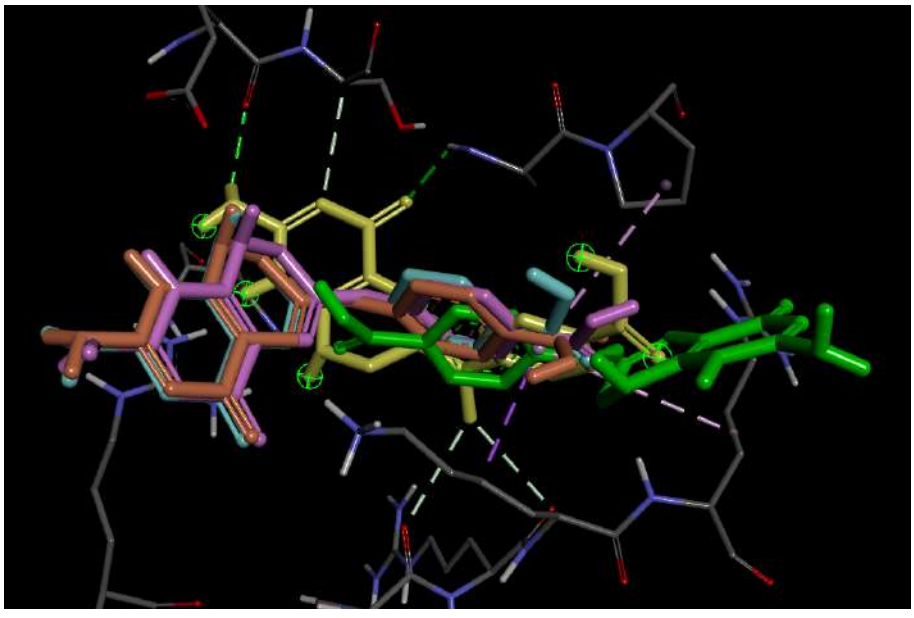


Figure 3.7 Comparing the similarity amongst Ligand 14 binding to different type of enzymes

Ligand 17

Table 3.8 RMSD value of ligand 17

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	2.45	0			
6JWW	4.34	4.02	0		
6JWZ	4.32	4.03	0.41	0	
6KCL	4.38	4.02	0.47	0.59	0

Positions of all ligand binding with receptor configurations are slightly different amongst themselves, except the WT receptor 6JWU as a result of RMSD value. Moreover, all positions of mutant ligand binding with receptor configurations are not significantly different from WT ligand (6JWU). However, the RMSD value indicates that 17_6JWV has the closest binding site to 14_6JWU (WT) with the result of 2.45 Å.

Remarkably, the RMSD value of another three mutant ligand binding with receptor configurations- 17_6JWW, 17_6JWZ, 17_6KCL- are very close to each other. According to the result, the RMSD value between 17_6JWW and 17_6JWZ is 0.41 Å. The RMSD value between 17_6JWW and 17_6KCL is 0.47 Å. Lastly, the RMSD value between 17_6JWZ and 17_6KCL is 0.59 Å. From the following data, it is eligible to conclude that these all three compounds possibly have similar binding sites, and therefore a similar interaction and activity since all RMSD values are less than 2.00 Å. To conclude, there is no ligand binding with mutants validates in this case since all of the binding results have RMSD value exceeding 2.00 Å.

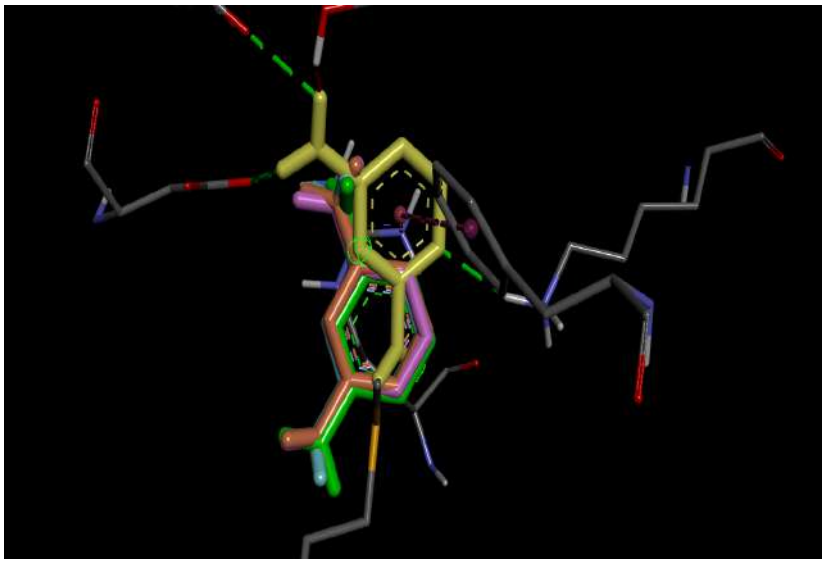


Figure 3.8 Comparing the similarity amongst Ligand 17 binding to different type of enzymes

Ligand 19

Table 3.9 RMSD value of ligand 19

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	7.68	0			
6JWW	7.53	1.39	0		
6JWZ	10.19	4.26	4.32	0	
6KCL	8.70	12.12	12.55	14.26	0

According to the result, 19_6JWW has the lowest RMSD value amongst all the ligand binding with mutant receptor configurations with the value 7.53 Å. From the data, 19_6JWV particularly resembles 19_6JWW in respect of the binding position as the RMSD between them is 1.39 Å, reportedly. In addition, 19_6JWZ has the most distinctive difference in binding position with the WT, reference to the RMSD 10.19 Å.

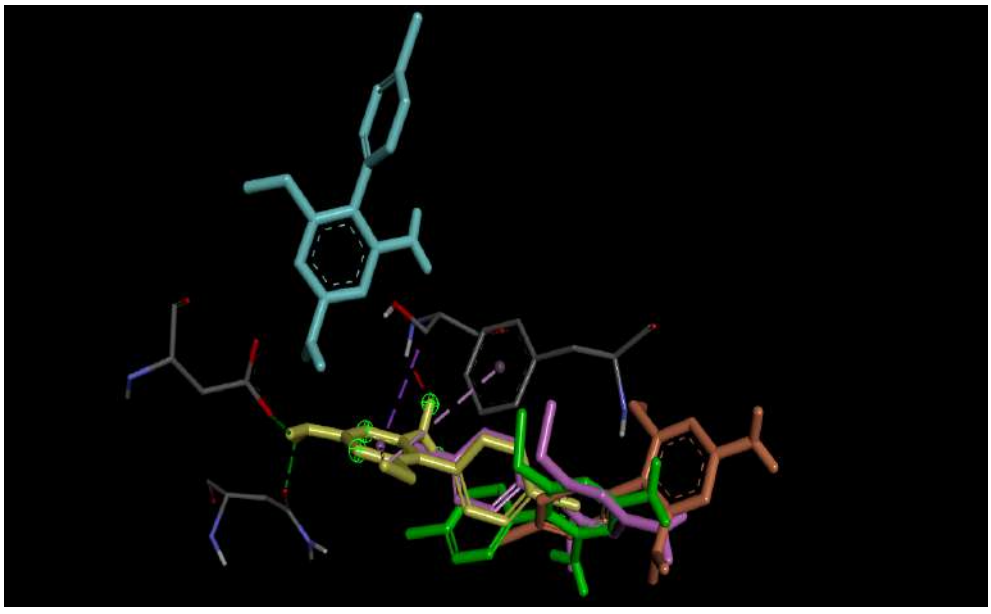


Figure 3.9 Comparing the similarity amongst Ligand 19 binding to different type of enzymes

3.1.1.2 Subgroup 1B

In this subgroup, ligands with docked configurations to mutants are all different to each other. In other words, there is no similarity amongst the ligands binding with mutant receptors in terms of the RMSD value. There are 5 compounds in this subgroup.

SDX-DHP

Table 3.10 RMSD value of ligand SDX-DHP

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	3.45	0			
6JWW	6.05	7.39	0		
6JWZ	8.50	8.78	10.54	0	
6KCL	3.55	2.51	8.10	7.83	0

According to the result, 6JWV_SDX-DHP has the closest binding position with WT, with an RMSD value of 3.45 Å. However, the RMSD value of either 6JWV_SDX-DHP or 6KCL_SDX-DHP compared with the WT are comparable to one another, as the value of 6KCL is 3.55 Å. Nonetheless, the RMSD value between both aforementioned configurations is slightly higher than the standard value of 2.00 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 6JWZ_SDX-DHP, with the RMSD value of 8.50 Å.

Figure 3.10 Comparing the similarity amongst SDX-DHP binding to different type of enzymes

STZ-DHP

Table 3.11 RMSD value of STZ-DHP

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	3.98	0			
6JWW	8.90	9.84	0		
6JWZ	8.73	9.16	6.24	0	
6KCL	3.94	2.50	10.15	8.77	0

According to the result, 6KCL_STZ-DHP has the closest binding position with 6JWU_STZ-DHP (WT), with an RMSD value of 3.94 Å. However, the RMSD value of either 6JWV_STZ-DHP or 6KCL_STZ-DHP compared with the WT are comparable to one another, as the value of 6KCL is 3.98 Å. Nonetheless, the RMSD value between both aforementioned configurations is slightly higher than the standard value, 2.00 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 6JWW_STZ-DHP, with the RMSD value of 8.90 Å.

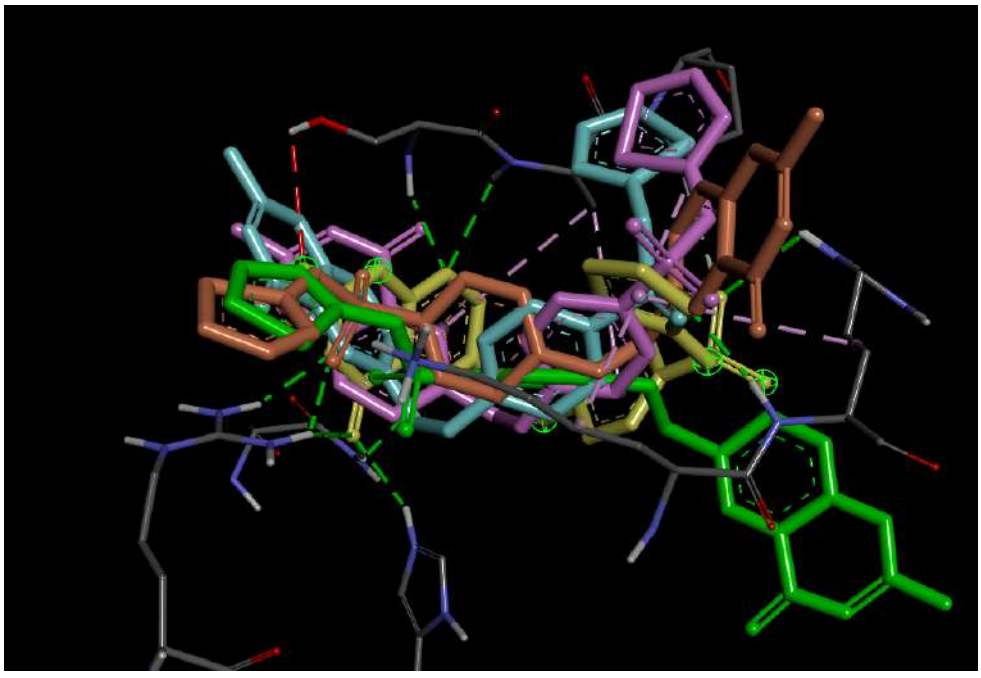


Figure 3.11 Comparing the similarity amongst STZ-DHP binding to different type of enzymes

PTA

Table 3.12 RMSD value of PTA

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	4.62	0			
6JWW	9.27	9.34	0		
6JWZ	8.75	8.47	2.80	0	
6KCL	5.13	0.99	9.71	8.79	0

Positions of all ligand binding with mutant receptor configurations are clearly different from one another. Likewise, the configurations of all ligands binding with mutants are totally different from WT (6JWU). According to the RMSD value, 6JWV has the closest binding position with WT ligand when comparing with other ligands, with the RMSD value of 4.62 Å, which is the smallest value amongst others. In addition, 6JWW_Pteroic Acid has the greatest dissimilarity in binding position amongst others in the report of RMSD value 9.27 Å.

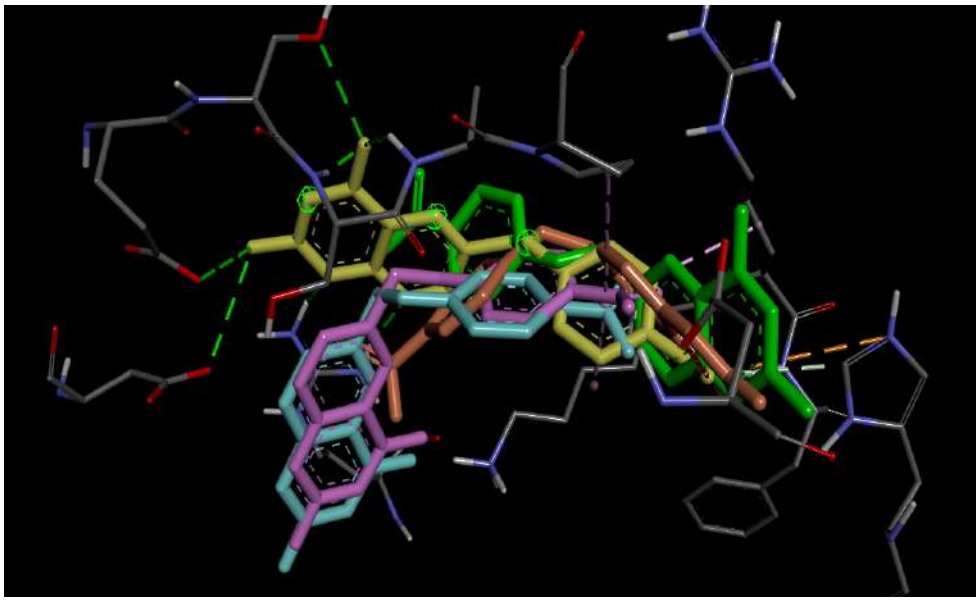


Figure 3.12 Comparing the similarity amongst PTA binding to different type of enzymes

Ligand 1

Table 3.13 RMSD value of Ligand 1

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	11.39	0			
6JWW	13.39	4.62	0		
6JWZ	6.05	9.48	11.73	0	
6KCL	5.97	9.01	11.15	5.95	0

According to the RMSD value, 6KCL has the closest binding position with WT ligand when comparing with other ligands, with the RMSD value of 5.97 Å, which is the smallest value amongst others. As a report of the data, 1_6JWZ position is very similar to 1_6KCL, with the RMSD value of 5.95 Å. In addition, 1_6JWW has the greatest dissimilarity in binding position amongst others in the report of RMSD value 13.39 Å.

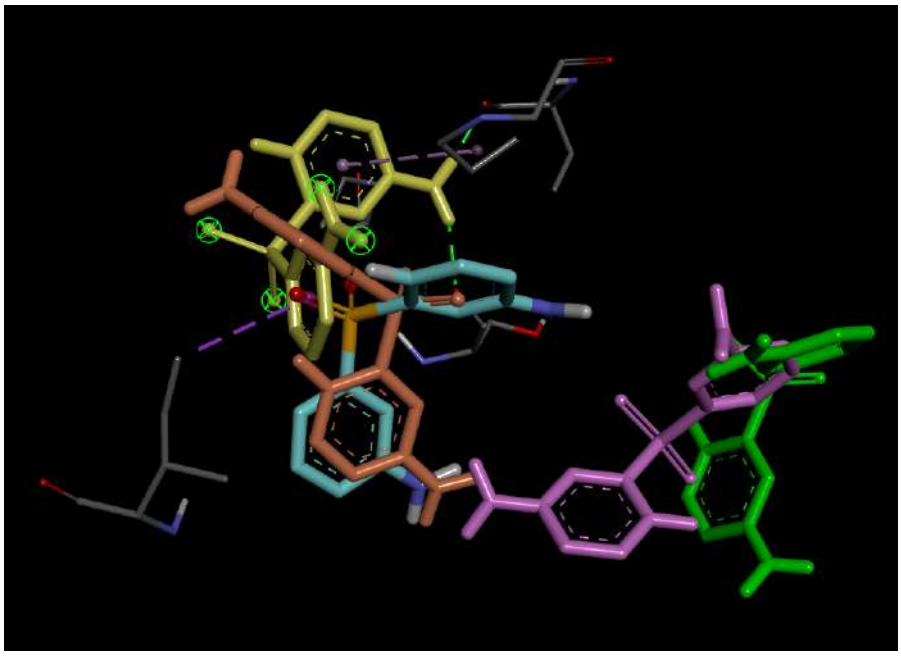


Figure 3.13 Comparing the similarity amongst ligand 1 binding to different type of enzymes

Ligand 13

Table 3.14 RMSD value of Ligand 13

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	11.53	0			
6JWW	7.68	13.79	0		
6JWZ	5.81	12.94	6.40	0	
6KCL	3.25	10.76	7.33	6.13	0

According to the result, 6KCL_Ligand number 13 has the closest binding site with 13_6JWU (WT), due to the RMSD value 3.25 Å. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 13_6JWV, with the RMSD value 11.53 Å.

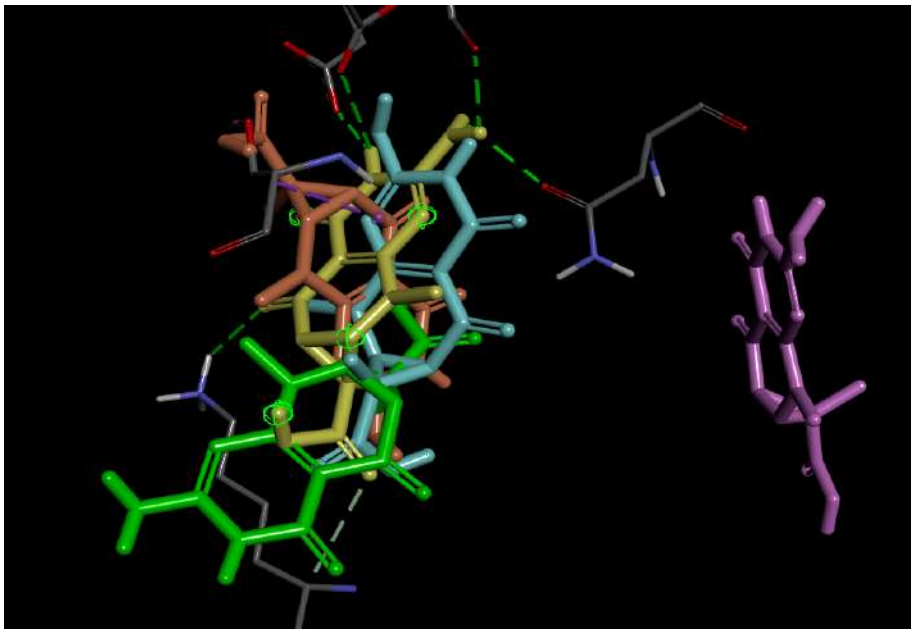


Figure 3.14 Comparing the similarity amongst ligand 13 binding to different type of enzymes

3.1.2 Ligands with binding mode in one mutant similar to that in WT

Ligands in this group (Group 2) have binding mode in only one mutant similar to that in WT, while the other 3 mutants are different from the WT. This group contains 4 compounds.

Ligand 2

Table 3.15 RMSD value of Ligand 2

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.10	0			
6JWW	6.97	8.87	0		
6JWZ	1.31	4.51	7.46	0	
6KCL	2.96	2.97	7.79	2.14	0

There is only a ligand binding with mutant, 6JWZ, has the most associated with WT, thus having the closest similarity with the original due to the RMSD value, 1.31 Å. It is important to note that with RMSD value below 2.00 Å means that two compounds have a very close binding site between them, and; therefore, mostly identical in interaction and activity. In contrast, the ligand binding with mutant receptor configuration that has the most significant dissimilarity with the WT is 2_6JWW, with the RMSD value 6.97 Å.

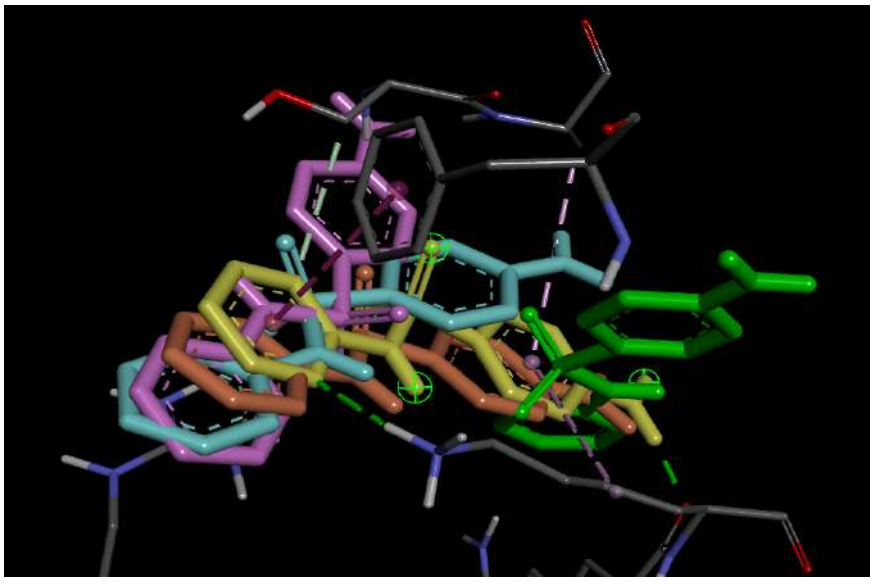


Figure 3.15 Comparing the similarity amongst ligand 2 binding to different type of enzymes

Ligand 6

Table 3.16 RMSD value of Ligand 6

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	5.76	0			
6JWW	1.52	6.44	0		
6JWZ	6.79	2.61	7.65	0	
6KCL	5.96	2.16	6.76	1.42	0

There is only a ligand binding with mutant, 6_6JWW, has the most associated with WT, thus having the closest similarity with the original in terms of interaction in accordance with the RMSD value, 1.52 Å. As the value is less than 2.00 Å, it is eligible to interpret that 6_6JWW is very similar to the WT in terms of binding site, therefore a similar interaction and activity. On the contrary, 6_6JWZ has the greatest disparity with the WT as the RMSD value is reportedly 6.79 Å.

In addition, 6_6JWZ has a proximate similarity to 6_6KCL in respect of the binding position, according to a report of the RMSD value, 1.42 Å. Furthermore, 6_6JWV insignificantly has the RMSD value out of the standard range, 2.00 Å, compared to 6_6KCL, with the value 2.16 Å.

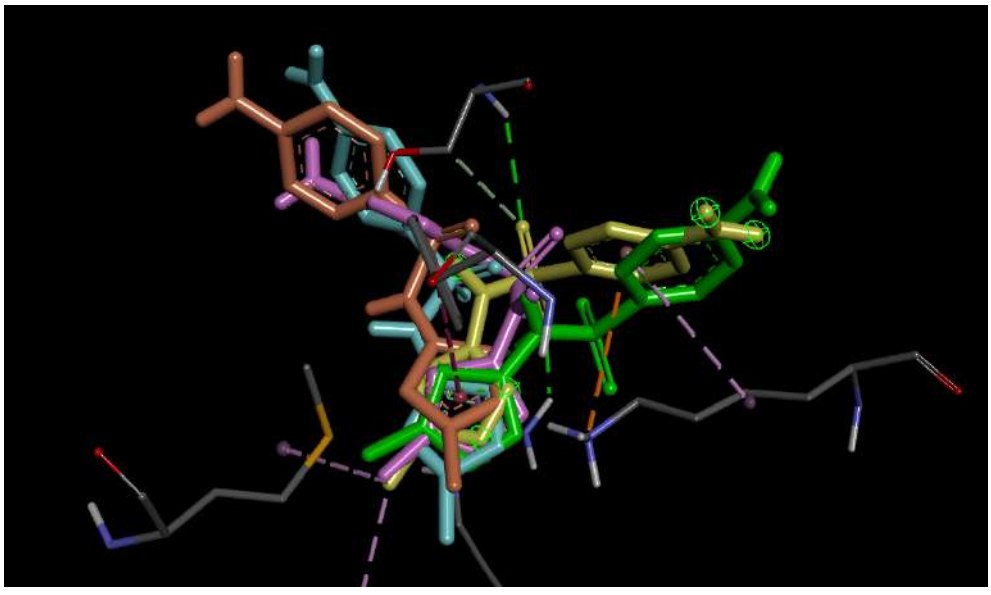


Figure 3.16 Comparing the similarity amongst ligand 6 binding to different type of enzymes

Ligand 8

Table 3.17 RMSD value of Ligand 8

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	3.44	0			
6JWW	1.12	3.57	0		
6JWZ	6.93	7.07	6.87	0	
6KCL	6.78	6.65	6.67	0.75	0

There is only a ligand binding with mutant, 8_6JWW, has the most associated with WT, thus having the closest similarity with the original as the result of the RMSD value, 1.12 Å. As the value is less than 2.00 Å, it is eligible to interpret that 8_6JWW is very similar to the WT in terms of binding site, therefore a similar interaction and activity. On the contrary, 8_6JWZ has the greatest disparity with the WT as the RMSD value is reportedly 6.93 Å.

In addition, 8_6JWZ has a proximate similarity to 8_6KCL in respect of the binding position, according to a report of the RMSD value, 0.75 Å.

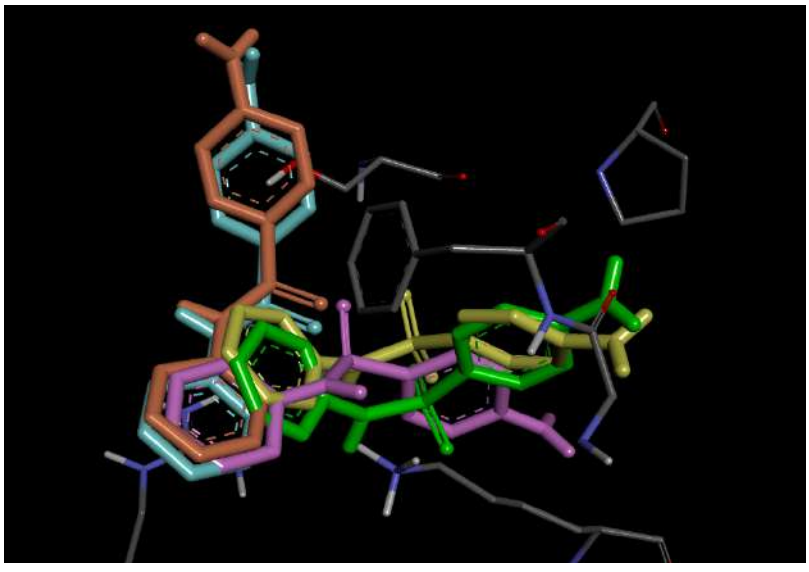


Figure 3.17 Comparing the similarity amongst ligand 8 binding to different type of enzymes

Ligand 9

Table 3.18 RMSD value of Ligand 9

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	10.06	0			
6JWW	1.84	9.51	0		
6JWZ	10.11	0.69	9.56	0	
6KCL	10.32	0.43	9.78	0.78	0

There is only a ligand binding with mutant, 9_6JWW, has the most associated with WT, thus having the closest similarity with the prototype as the report of the RMSD value, 1.84 Å. As the value is less than 2.00 Å, it is eligible to interpret that 9_6JWW is very similar to the WT in terms of binding site, therefore a similar interaction and activity. On the contrary, 9_6KCL has the greatest disparity with the WT as the RMSD value is reportedly 10.32 Å.

Interestingly, the RMSD value of another three mutant ligand binding with receptor configurations- 9_6JWV, 9_6JWZ, 9_6KCL- are extremely close to each other. According to the result, the RMSD value between 9_6JWV and 9_6JWZ is 0.69 Å. The RMSD value between 9_6JWV and 9_6KCL is 0.43 Å. Lastly, the RMSD value between 9_6JWZ and 9_6KCL is 0.78 Å. From the following data, it is eligible to conclude that these all three compounds possibly have similar binding sites, and therefore a similar interaction and activity since all RMSD values are less than 2.00 Å.

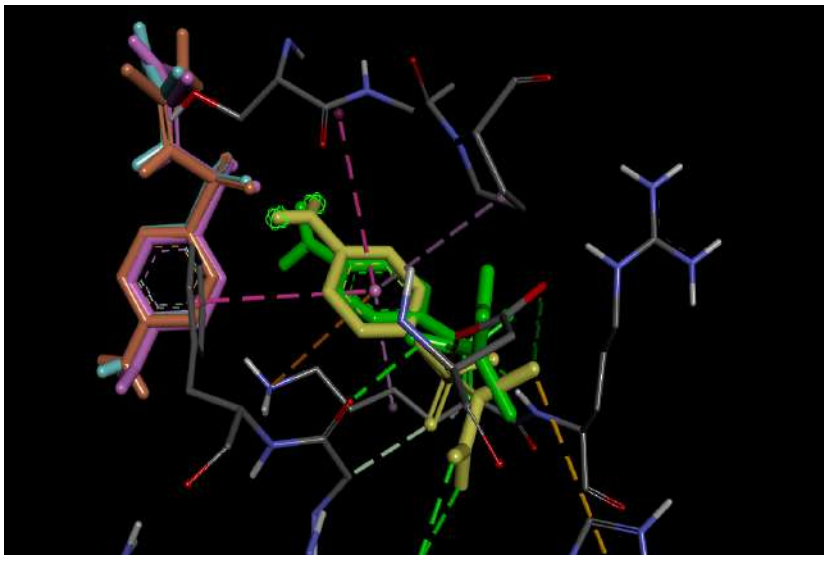


Figure 3.18 Comparing the similarity amongst ligand 9 binding to different type of enzymes

3.1.3 Ligands with binding modes in two mutants similar to that in WT

Ligands in this group (Group 3) have binding modes in two mutants similar to that in WT, while the other 2 mutants are different from the WT. This group contains 3 compounds.

Ligand 5

Table 3.19 RMSD value of Ligand 5

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	0.42	0			
6JWW	6.53	6.53	0		
6JWZ	0.66	0.87	6.54	0	
6KCL	7.06	7.22	8.65	6.78	0

5_6JWV and 5_6JWZ are very highly similar with WT in terms of binding site regarding the RMSD value, 0.42 Å and 0.66 Å, respectively. As either of those configurations have the RMSD value lower than 2.00 Å when compared to 5_6JWV, it is acceptable to presume that they have mostly the same interaction and activity with the WT. This also means that both 5_6JWV and 5_6JWZ are significantly similar to one another. According to the result, the RMSD value between 5_6JWV and 5_6JWZ is 0.87 Å. On the contrary, 5_6KCL has the greatest disparity with the WT as the RMSD value is reportedly 7.06 Å.

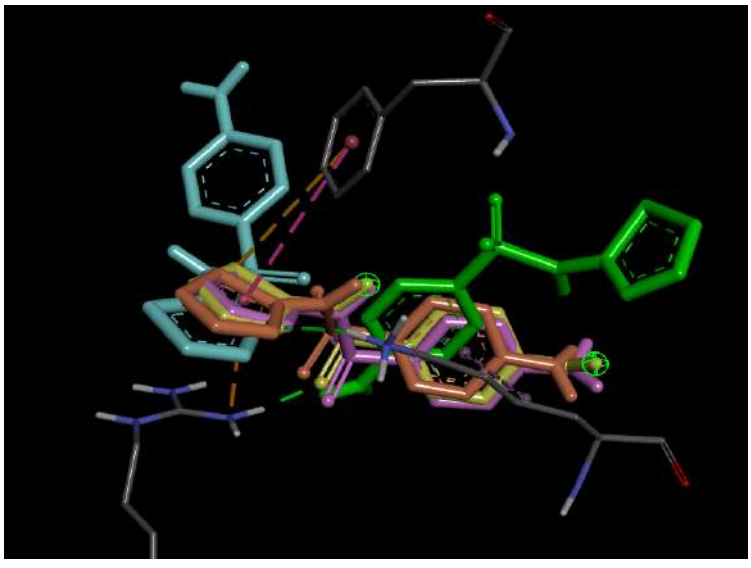


Figure 3.19 Comparing the similarity amongst ligand 5 binding to different type of enzymes

Ligand 16

Table 3.20 RMSD value of Ligand 16

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	0.34	0			
6JWW	3.31	3.09	0		
6JWZ	25.91	25.81	26.05	0	
6KCL	0.50	0.64	3.48	25.84	0

16_6JWV and 16_6KCL are very highly similar with WT in terms of binding site regarding the RMSD value, 0.34 Å and 0.50 Å, respectively. As either of those configurations have the RMSD value lower than 2.00 Å when compared to 16_6JWV, it is acceptable to presume that they have mostly the same interaction and activity with the WT. This also means that both 16_6JWV and 16_6KCL number 16 are significantly similar to one another. According to the result, the RMSD value between 16_6JWV and 16_6KCL is 0.64 Å. On the contrary, 6JWZ_Ligand number 16 has the greatest disparity with the WT as the RMSD value is reportedly 25.91 Å.

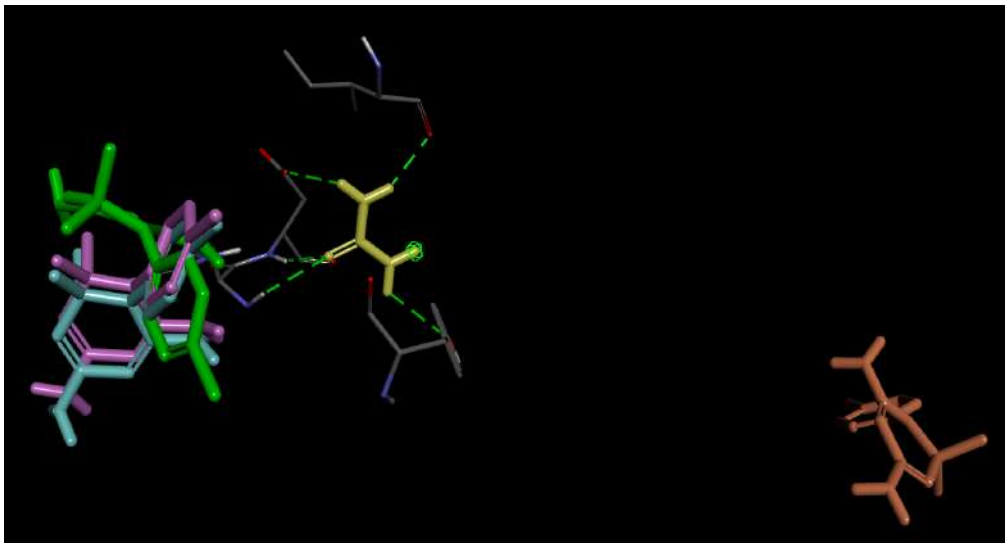


Figure 3.20 Comparing the similarity amongst ligand 16 binding to different type of enzymes

Ligand 18

Table 3.21 RMSD value of Ligand 18

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	0.31	0			
6JWW	8.64	8.67	0		
6JWZ	23.05	23.07	22.52	0	
6KCL	0.34	0.39	8.63	22.87	0

18_6JWV and 18_6KCL are very highly similar with WT in terms of binding site regarding the RMSD value, 0.31 Å and 0.34 Å, respectively. As either of those configurations have the RMSD value lower than 2.00 Å when compared to 18_6JWV, it is acceptable to presume that they have mostly the same interaction and activity with the WT. This also means that both 18_6JWV and 18_6KCL are significantly similar to one another. According to the result, the RMSD value between 18_6JWVand 18_6KCL is 0.39 Å. On the contrary, 18_6JWZ has the greatest disparity with the WT as the RMSD value is reportedly 23.05 Å.

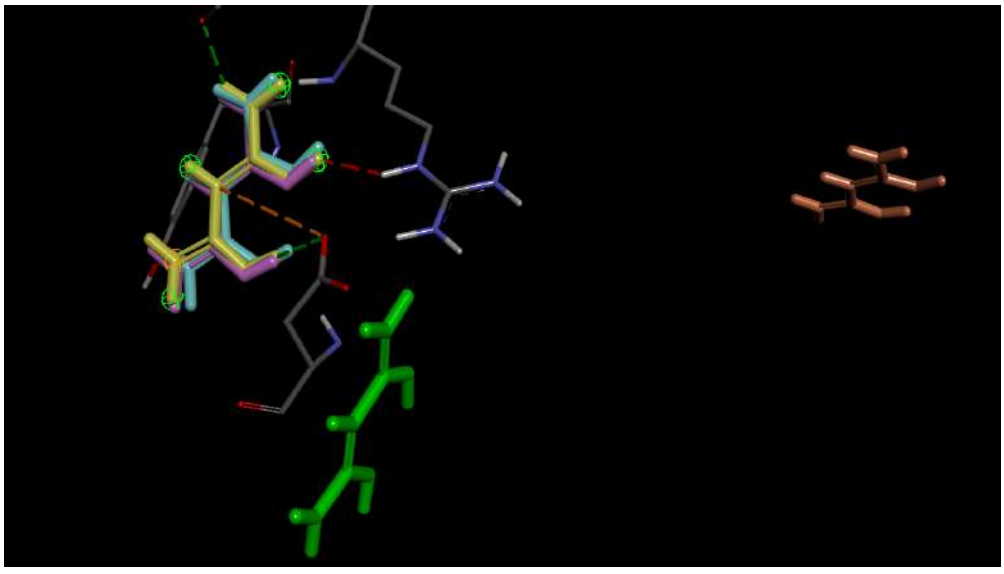


Figure 3.21 Comparing the similarity amongst ligand 18 binding to different type of enzymes

3.1.4 Ligand with binding modes in three mutants similar to that in WT

Ligand in this group (Group 4) has binding modes in three mutants similar to that in WT, while the other 1 mutant is different from the WT. There is only 1 ligand in this group.

Ligand 15

Table 3.22 RMSD value of Ligand 15

Enzyme	6JWU	6JWV	6JWW	6JWZ	6KCL
6JWU	0				
6JWV	2.03	0			
6JWW	1.50	2.42	0		
6JWZ	2.00	0.48	2.20	0	
6KCL	0.55	2.05	1.02	1.96	0

Positions of all ligand binding with mutant receptor configurations are very much alike amongst themselves and also the WT. According to the data, 15_6KCL has the closest binding site with WT as a consequence of the RMSD value 0.55 Å, reportedly. As the value is quite near zero, it indicates that 15_6KCL is highly alike with WT in respect of binding position, thus having significantly similar interaction and activity to the original.

Remarkably, the rest of the three ligands binding with mutant receptors- 15_6JWV, 15_6JWW, and 15_6JWZ- are all having the RMSD value correlate with 15_6JWU in the range of 2.00 Å, approximately. The RMSD of those aforementioned configurations are 2.03 Å, 1.50 Å, and 2.00 Å, respectively.

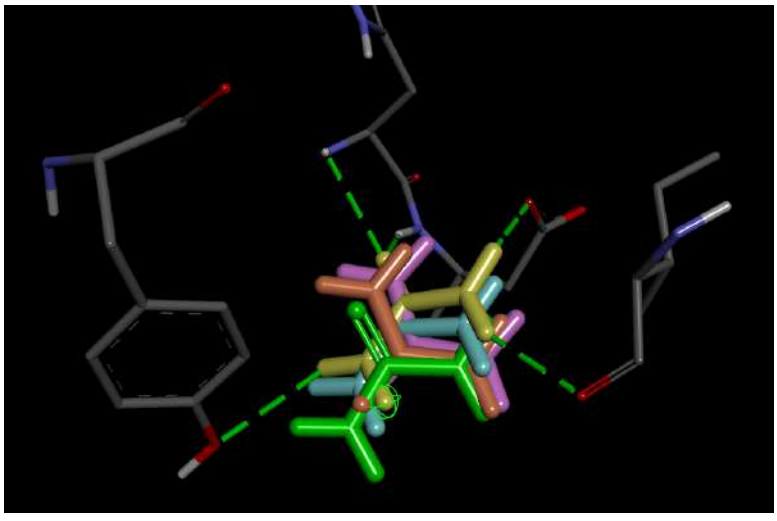


Figure 3.22 Comparing the similarity amongst ligand 15 binding to different type of enzymes

3.2 Enzyme-ligand interactions

In this part, interactions of the three inhibitors to the WT (6JWU) and the four mutants (6JWV, 6JWW, 6JWZ, and 6KCL) were comparatively analyzed. Then, key binding residues for activity in both WT and mutants were identified. Ligands that bind to these key binding residues in each enzyme are probably active against this enzyme.

3.2.1 Identifying key binding residue

According to the result of docking, SDX-DHP binding with WT enzyme has interaction with amino acids HIS at 688, ASN at 396, SER at 436, ALA at 437, LYS at 609, ARG at 610, PRO at 438, and ARG at 686. However, there are certain amino acids in all four mutant enzymes (6JWV, 6JWW, 6JWZ, and 6KCL) that are not able to be found when compared to their derivative. In 6JWV binding with the inhibitor SDX-DHP (6JWV_SDX-DHP), there is no interaction with amino acids HIS at 688, ASN at 396, SER at 436, ALA at 437, and PRO at 438. In 6JWW_SDX-DHP, there is no interaction with amino acids SER at 436, ALA at 437, ARG at 610, and PRO at 438. In 6JWZ_SDX-DHP, there is no interaction with amino acid SER at 436, ALA 437, and PRO at 438. In 6KCL_SDX-DHP, there is no interaction with amino acids ASN at 396, SER at 436, ALA at 437, and PRO at 438.

According to the result of docking, STZ-DHP binding with a WT enzyme has interaction with amino acids exactly the same as SDX-DHP inhibitor. Similarly, there are certain amino acids in all four mutant enzymes- 6JWV, 6JWW, 6JWZ, and 6KCL- that are not able to be found when compared to their derivative. In 6JWV binding with the inhibitor STZ-DHP, there is no interaction with amino acids same as the inhibitor SDX-DHP. There is similarity between 6JWW_SDX-DHP and 6JWW_STZ-DHP in terms of the absence of interaction with certain amino acids, but with one additional not present amino acid, ASN at 396. In 6JWZ_SDX-DHP, there is no interaction with amino acid HIS at 688, ASN at 396, SER at 436, ALA 437, and PRO at 438. In 6KCL_STZ-DHP, there is no interaction with amino acids HIS at 688, ASN at 396, SER at 436, ALA at 437, and PRO at 438.

To identify the key residues of the interaction, both SDX-DHP and STZ-DHP were compared to the inhibitor PTA to determine the key residues of the interaction as it could be either performed with WT enzyme or mutant enzyme while sulfa drugs could only be active with the derivative. As a result, only two key residues were found in all receptors which are the amino acids ARG at 686 and LYS at 609. It is important to note that 6JWU and 6JWR are the same category of enzyme but with different binding with the inhibitor. Likewise, 6JWV and 6JWS belong to the same equivalence class as well as 6JWW and 6JWT.

	No.	HIS 688	ASN 396	SER 436	ALA 437	LYS 609	ARG 610	PRO 438	ARG 686	PRO 442	PRO 535
2	SDX-DHP										
3	6JWU	✓	✓	✓	✓	✓	✓	✓	✓		
4	6JWV					✓	✓		✓		
5	6JWW	✓	✓			✓			✓		
6	6JWZ	✓	✓			✓	✓		✓		
7	6KCL	✓				✓	✓		✓		
8											
9	STZ-DHP										
10	6JWU	✓	✓	✓	✓	✓	✓	✓	✓		
11	6JWV					✓	✓		✓		
12	6JWW	✓				✓	✓		✓		
13	6JWZ					✓	✓		✓		
14	6KCL					✓	✓		✓		
15											
16	PTA										
17	6JWU			✓	✓	✓	✓	✓	✓		
18	6JWV					✓	✓		✓		
19	6JWW	✓	✓			✓	✓		✓		
20	6JWZ					✓	✓		✓		
21	6KCL					✓	✓		✓		
22											

Figure 3.23 Identifying the key binding residues

Both SDX-DHP and STZ-DHP are significantly similar to one another on the fact that either of the inhibitors have the same key amino acid interaction. From Figure 3.24, the structures of both inhibitors are greatly similar to each other. The amino acid PRO at the residue 438 could be found only in the WT as it is completely absent in all mutants, according to the report of the data.

To clarify the configurations in Figure 3.24, the yellow color represents the inhibitor SDX-DHP and the pink color represents STZ-DHP.

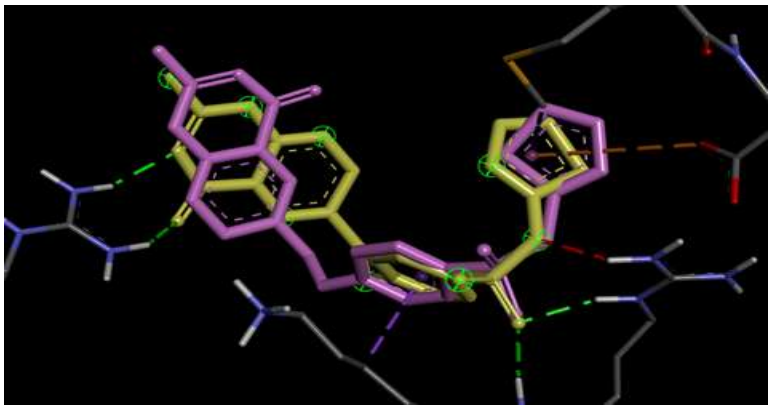


Figure 3.24 Comparing SDX-DHP to STZ-DHP

According to the X-ray verification, PTA inhibitor binding with the WT enzyme (6JWR) has interaction with amino acids SER at 436, ALA at 437, LYS at 609, ARG at 610, PRO at 438, ARG at 686, PHE at 580, PHE at 603, ASP at 482, LEU at 578, GLY at 605, MET at 538, VAL at 527, GLY at 777, ASP at 575, ILE at 504, and ASN at 502. Comparing the mutant 6JWS to the WT, the interaction with amino acids are not found in certain residues including ALA at 437 and LEU at 578. For 6JWT, there is also the absence of certain amino acids including SER at 436, ALA at 437, LEU at 578. Currently, there is no publishing for the X-ray structures between the inhibitor PTA and the mutants 6JWZ and 6KCL.

There is high possibility that the sulfa drugs, SDX-DHP and STZ-DHP, are inactive with the mutant enzymes because of the absence of the interaction with amino acid PRO at the residue 438; due to the fact that the aforementioned amino acid interaction could be found in the inhibitor PTA binding with any types of enzymes, while this interaction with sulfa drugs only occurs in the WT class.

	A	B	C	D	E	F	G	H	I	J
1	No.	HIS 688	ASN 396	SER 436	ALA 437	LYS 609	ARG 610	PRO 438	ARG 686	PRO 442
2	SDX-DHP									
3	6JWZ (x-ray)					✓	✓		✓	
4										
5	STZ-DHP									
6	6JWU (x-ray)					✓	✓	✓	✓	
7	6JWV (x-ray)					✓	✓		✓	
8	6JWW (x-ray)					✓	✓		✓	
9										
10	PTA (x-ray)									
11	6JWR			✓	✓	✓	✓	✓	✓	
12	6JWS			✓		✓	✓	✓	✓	
13	6JWT					✓	✓	✓	✓	
14										

Figure 3.25 The interaction PRO at 438 in X-ray structures

In Figure 3.26, interaction PRO at the residue 438 was detected in the inhibitor PTA binding to the 6JWS. On the contrary, in Figure 3.27, the interaction is not found in STZ-DHP, the sulfa-inhibitor, binding to the identical enzyme. The aforementioned interaction could only be found in the WT enzyme binding to either STZ-DHP or PTA. However, the presumption is required for further examination as the X-ray structure of SDZ-DHP is currently not available in a database, Protein Data Bank; as well as other unavailable structures including SDX-DHP with 6JWV, 6JWW and 6KCL, STZ-DHP with 6JWZ and 6KCL, and lastly PTA with 6JWZ and 6KCL.

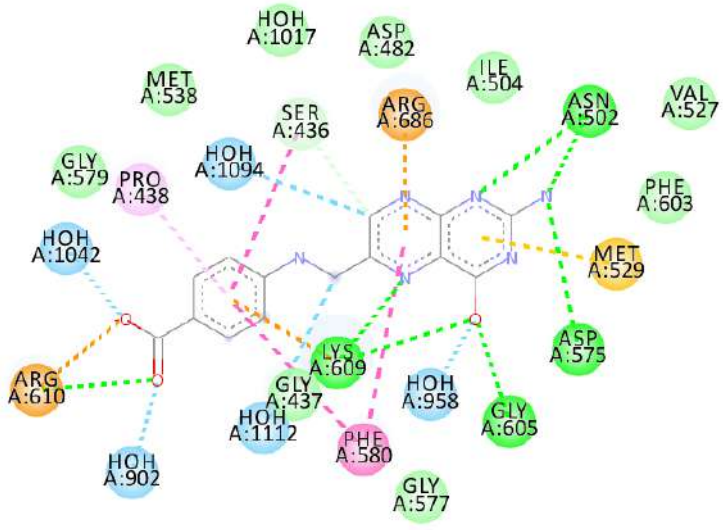


Figure 3.26 6JWS binding to PTA (X-ray structure)

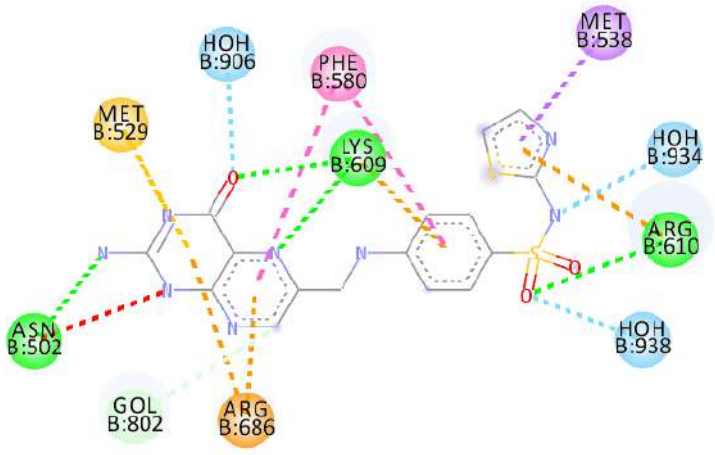


Figure 3.27 6JWV binding to STZ-DHP (X-ray structure)

3.2.2 Identifying active ligands to each enzyme

Comparing the docked configuration amongst all kinds of ligands, sulfa inhibitors, and PTA inhibitor binding to the WT enzyme, certain of the ligands have fairly similar amino acid interaction with those three inhibitors, including ligand number 8 and 14. Besides, the rest of ligands are distinguishable from the inhibitors. According to the docking result, ligand number 8 binding to 6JWU enzyme has interaction with amino acids SER at 436, LYS at 609, PRO at 438, ARG at 686, and PHE at 580, in which LYS at 609 and ARG 686 are the key residues. Ligand number 14 binding to the WT enzyme has interaction with amino acids ASN at 396, ALA at 437, LYS at 609, ARG at 610, PRO at 438, ARG at 686, and PHE at 580, where the amino acid ASN at 396 solely appears in sulfa inhibitors.

Comparing the docked configuration amongst all kinds of ligands, sulfa inhibitors, and PTA inhibitor binding to the 6JWV enzyme, certain of the ligands have fairly similar amino acid interaction with those three inhibitors, including ligand number 1, 2, 5, 6, 7, 8, 10, 12, and 14. However, none of these ligands has interaction with amino acid PRO at the residue 438.

Comparing the docked configuration amongst all kinds of ligands with sulfa inhibitors inhibitor binding to the 6JWW enzyme, certain of the ligands have fairly similar amino acid interaction with those three inhibitors, including ligand number 1, 4, 6, 8, 10, 11, 12, and 14. Remarkably, only ligand 11 binding to the mutant enzyme has an interaction with amino acid PRO at the residue 438.

Comparing the docked configuration amongst all kinds of ligands with sulfa inhibitors inhibitor binding to the 6JWZ enzyme, certain of the ligands have fairly similar amino acid interaction with those three inhibitors, including ligand number 2, 5, 10, 12, and 14. Nevertheless, none of these ligands has interaction with amino acid PRO at the residue 438.

Comparing the docked configuration amongst all kinds of ligands with sulfa inhibitors inhibitor binding to the 6KCL enzyme, certain of the ligands have fairly similar amino acid interaction with those three inhibitors, including ligand number 2, 6, 7, 10, and 14. Nonetheless, none of these ligands has interaction with amino acid PRO at the residue 438.

In summary, only Ligand 14 was considered to be active with the mutants as the ligand binding to each enzyme - WT, 6JWV, 6JWW, 6JWZ, and 6KCL - has interaction with key binding residue including ARG at 686 and LYS at 609.

Last, but not least, this is important to note that all of the 2D diagrams of each ligand interaction were provided at the Appendix A - Table A.

Ligand 14

This ligand might possibly be active with every enzyme due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWU enzyme has interaction with amino acids LYS at 609, ARG at 686, ASN at 396, ALA at 437, ARG at 610, PRO at 438, SER at 435, PHE at 580, GLU at 434, and SER at 607. The ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 610, GLY at 579, PHE at 580, MET at 529, ASN at 502, and ASP at 575. The ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 610, GLY at 579, ASP at 539, HIS at 584, and LYS at 582. The ligand binding to the 6JWZ enzyme has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, MET at 529, ASP at 575, GLY at 605, and ASN at 502. The ligand binding to the 6KCL enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 610, PHE at 580, MET at 529, ASP at 575, GLY at 605, and ASN at 502.

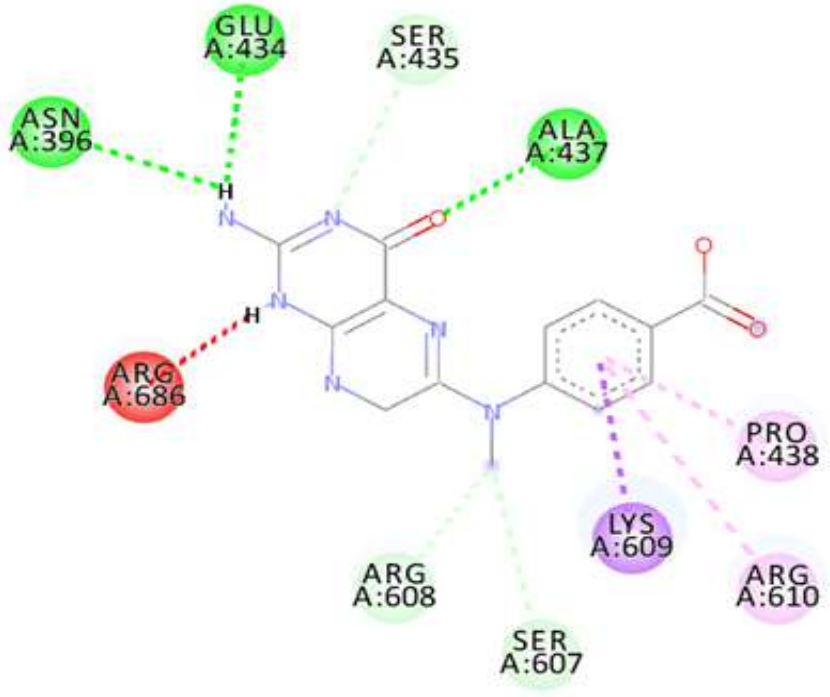


Figure 3.28 6JWU binding to Ligand 14

According to the data, the ligand binding to the WT has related similarity to the inhibitor PTA binding to the same receptor terms of amino acid interactions including LYS at 609, ARG at 686, ARG at 610, PRO at 438, SER at 435, and GLU at 434. Likewise, the ligand binding to the mutant 6JWV has certain associations in terms of the amino acid interactions with the inhibitor PTA binding to the mutant 6JWV including LYS at 609, ARG at 686, ARG at 610, GLY at 579, and PHE at 580. The ligand binding to the mutant 6JWW has certain associations in terms of the amino acid interactions with the inhibitor PTA binding

to the mutant 6JWW including LYS at 609, ARG at 686, and ARG at 610. The ligand binding to the mutant 6JWZ has certain associations in terms of the amino acid interactions with the inhibitor PTA binding to the mutant 6JWZ including LYS at 609, ARG at 686, PHE at 580. MET 529, ASP at 575, GLY at 605, and ASN at 502. Lastly, the ligand binding to the mutant 6KCL has certain associations in terms of the amino acid interactions with the inhibitor PTA binding to the mutant 6KCL including LYS at 609, ARG at 686, ARG at 610, PHE at 580, GLY at 579, and ASP at 575.

To clarify the configurations in Figure 3.29, the yellow color represents the inhibitor PTA and the pink color represents Ligand 14.

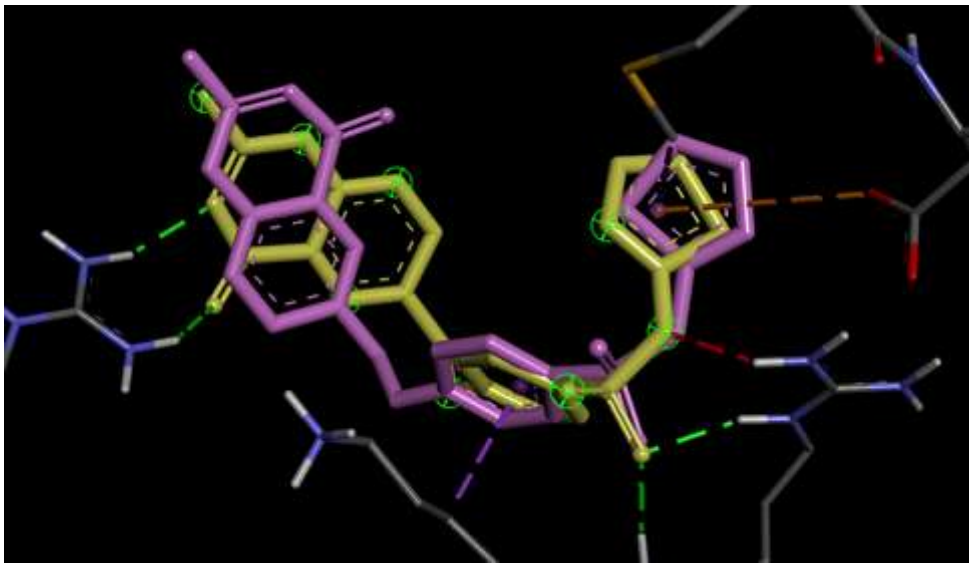


Figure 3.29 Comparing 6JWV_Ligand 14 to 6JWV_Pteroic Acid

Ligand 1

This ligand might possibly be active with the mutant enzymes 6JWV and 6JWW due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. The ligand binding to 6JWV has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, GLY at 579, and ASP at 482, meanwhile the ligand binding to 6JWW has interaction with amino acids LYS at 609, ARG at 686, HIS at 688, GLY at 579, SER at 607, PHE 436, and GLY at 437.

Ligand 2

This ligand might possibly be active with the WT enzyme, 6JWV enzyme, and 6JWZ enzyme due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the docking result, the ligand binding to the enzyme 6JWU has interaction with amino acids LYS at 609, ARG at 686, SER at 436, ALA at 437, PRO at 535, VAL 440, SER at 435, and ILE at 484. The ligand binding to 6JWV has interaction with amino acids LYS at

609, ARG at 686, PHE at 580, GLU at 434, and MET at 529. Lastly, the ligand binding to 6JWZ has interaction with amino acids LYS at 609, ARG at 686, HIS at 688, ARG at 608, PHE at 580, and ASP at 482.

Ligand 3

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 4

This ligand might possibly be active only with the mutant 6JWW due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, ASN at 396, SER at 607, and PHE at 436.

Ligand 5

This ligand might possibly be active with the WT enzyme and the mutant enzymes 6JWV and 6JWZ due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWU enzyme has interaction with amino acids LYS at 609, ARG at 686, and PHE at 580. The ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG a 686, ARG at 608, and PHE at 580. Lastly, the ligand binding to the 6JWZ enzyme has interaction with amino acids LYS at 609, ARG at 686, ILE at 484, and PHE at 580.

Ligand 6

This ligand might possibly be active with the mutant enzymes 6JWV, 6JWW, and 6KCL due to the interaction with the key residue amino acids LYS at 609 and ARG at 686.. According to the data, the ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, MET at 539, ASP at 482, ILE at 504. The ligand binding to the 6JWW enzyme has interaction with amino acids significantly close to 6JWV, except the absence of the interaction of the amino acid ASP at the residue 482. Lastly, The ligand binding to the 6KCL enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 608, SER at 435, PHE at 580, GLU at 434, PHE at 603, MET at 529, ASP at 483, and ILE at 504.

Ligand 7

This ligand might possibly be active with the WT, the mutants 6JWV and 6KCL due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWU enzyme has interaction with amino acids LYS at 609, ARG at 686, SER at 436, ALA at 437, ARG at 608, PHE at 580, and MET at 529. The

ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, PHE at 603, MET 529, and ILE 504. Lastly, the ligand binding to the 6KCL enzyme has interaction with amino acids LYS at 609, ARG at 686, SER at 435, PHE at 580, PHE at 603, MET at 529, and ILE at 504.

Ligand 8

This ligand might possibly be active with the WT, the mutants 6JWV and 6JWW due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWU enzyme has interaction with amino acids LYS at 609, ARG at 686, SER at 436, PRO at 438, ARG at 608, PHE at 580, and GLY at 579. The ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 608, PHE at 580, PHE at 603, MET 529, and ILE 504. Lastly, the ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, GLY at 579, and GLY at 437.

Ligand 9

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 10

Ligand 10 might possibly be active with every mutant enzyme including 6JWV, 6JWW, 6JWZ, and 6KCL. According to the data, the ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 608, PHE at 580, MET at 529, and ILE at 504. The ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, PHE at 580, GLY at 579, PHE at 436, and ILE at 394. The ligand binding to the 6JWZ enzyme has interaction with amino acids greatly similar to 6JWV enzyme, but with the adding amino acid interaction of HIS at the residue 688. Lastly, the ligand binding to the 6KCL enzymes has interaction with amino acids LYS at 609, ARG at 686, SER at 435, PHE at 580, GLU at 434, and ILE 504.

Ligand 11

This ligand might possibly be active only with the mutant 6JWW. According to the data, the ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, ARG at 610, and PHE at 580. Remarkably, the ligand also has the interaction with PRO at the residue 438, which generally is not presented in any ligand binding to mutants.

Ligand 12

This ligand might possibly be active with the mutants 6JWV, 6JWW, and 6JWZ due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. According to the data, the ligand binding to the 6JWV enzyme has interaction with amino acids LYS at 609, ARG at 686, ASN at 396, PHE at 580, MET at 529, ASP 482, ASP at 575, ILE at 504, GLY at 605, and ASN at 502. The ligand binding to the 6JWW enzyme has interaction with amino acids LYS at 609, ARG at 686, HIS at 688, ARG at 610, and GLY at 579. Lastly, the ligand binding to the 6JWZ enzyme has interaction with amino acids LYS at 609, ARG at 686, HIS at 688, PHE at 580, MET at 529, ASP at 482, ASP at 575, GLY at 605, and ASN 502.

Ligand 13

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 15

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 16

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 17

This ligand might possibly be active only with the WT enzyme. According to the data, the ligand binding to the 6JWU enzyme has interaction with amino acids LYS at 609, ARG at 686, SER at 436, PHE at 580, GLU at 434, MET at 529, and ASP at 482.

Ligand 18

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Ligand 19

This ligand was found inactive with every class of enzymes as a result of the absence of the interaction with both key amino acids including LYS at 609 and ARG at 686.

Although the interaction PRO at the residue 438 was not discovered in the ligand 14 binding to any mutants, however, the ligand might be found with X-ray crystallography experiment where the result is considerably more precise and reliable; due to the fact that the capability of computational molecular docking is limited with several factors. One of the factors is that the enzymes in our docking simulation are in rigid form as the binding site was adjusted to be fitted with all the ligands, in turns, unable to bend or be forced out of shape. Second, docking is a method which predicts that preferred orientation of one molecule to a second, when bound to each other, forms a stable complex in which either the position or conformation are randomly selected by the programme. On the contrary, X-ray crystallography is a technique by which it is possible to determine the three dimensional positions of each atom in a protein. As the ligand could alter its conformation on account of the flexible docking, the possibility of the result is almost infinite. Therefore, it is nearly impossible to receive the result exactly the same with the X-ray method.

This research could be used as a framework for further study of malaria resistant drug development. Although the PTA inhibitor is active in both WT and mutant enzymes, the compound tends to have poor solubility. Due to its planar structure, the compound is utterly more stable in a crystal lattice than in a solution form. To be effective, drugs must well dissolve into the body, therefore compounds with poor solubility have been found to not make good drugs. Moreover, the commercial antimalarial drugs that are available in the market cause several severe side effects to patients, for instance, nausea, vomiting, abdominal pain, headache, diarrhea, etc. As a result, the side effects of these drugs make the need for the necessity of new effective drugs.

The result could be more precise if further simulation of various X-ray structures of the inhibitor PTA binding to the mutants 6JWV and 6KCL are available; also the sulfa-inhibitor SDX-DHP binding to the WT, 6JWV, 6JWW, and 6KCL and STZ-DHP binding to 6JWZ and 6KCL. Moreover, the research would definitely be more accurate if the half-maximal inhibitory concentration (IC_{50}) of all ligands are published since the value provides a measure of a drug's efficiency.

Chapter 4

CONCLUSION

DHPS is an enzyme in the folate synthesis pathway; a crucial pathway for synthesizing amino acids. However, due to high clinical usage of the sulfa-drugs, parasitic populations have built up resistance to these drugs. Therefore, the main purpose was to determine the cause of the in-effectiveness of the sulfa inhibitors, SDX-DHP and SDZ-DHP, along with finding the potential ligands which are possibly active either with WT or mutants. Docking configurations of sulfa-drugs were compared to that of Pteroic acid (PTA), which is active in both WT and mutants.

From molecular docking calculations, there is a high possibility that the sulfa drugs, SDX-DHP and STZ-DHP, are inactive with the mutant enzymes because of the absence of the interaction with amino acid PRO at the residue 438; due to the fact that the aforementioned amino acid interaction could be found in the inhibitor PTA binding with any types of enzymes, while this interaction with sulfa drugs only occurs in the WT class. In addition, ligand 14 (7,8-dihydropteroic acid) are the ligands with a fair potential to be active with the mutants as certain amino acid interactions similar to the inhibitor PTA were found. According to the results, ligand 14 might possibly be active with every enzyme due to the interaction with the key residue amino acids LYS at 609 and ARG at 686. The ligand 14 binding to the WT has related similarity to the inhibitor PTA binding to the same receptor terms of amino acid interactions including LYS at 609, ARG at 686, ALA at 437, ARG at 610, PRO at 438, and PHE at 580. The ligand binding to the mutant 6JWV has certain associated in terms of the amino acid interactions with the inhibitor PTA binding to the mutant 6JWV including LYS at 609, ARG at 686, ARG at 610, GLY at 579, and PHE at 580. Likewise, the ligand binding to the mutant 6JWW has certain associated in terms of the amino acid interactions with the inhibitor PTA binding to the mutant 6JWW including LYS at 609, ARG at 686, ARG at 610, and GLY at 579.

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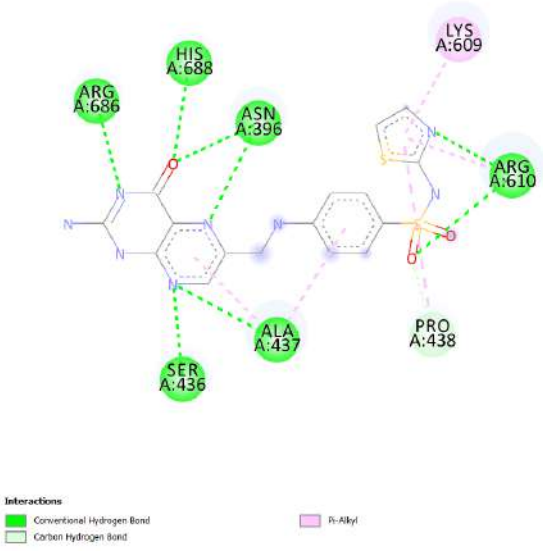
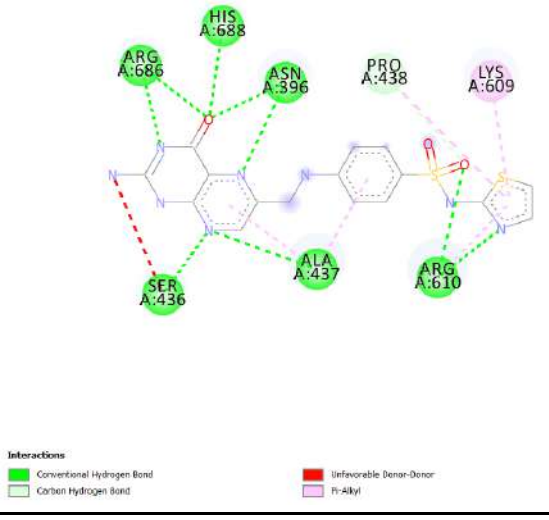
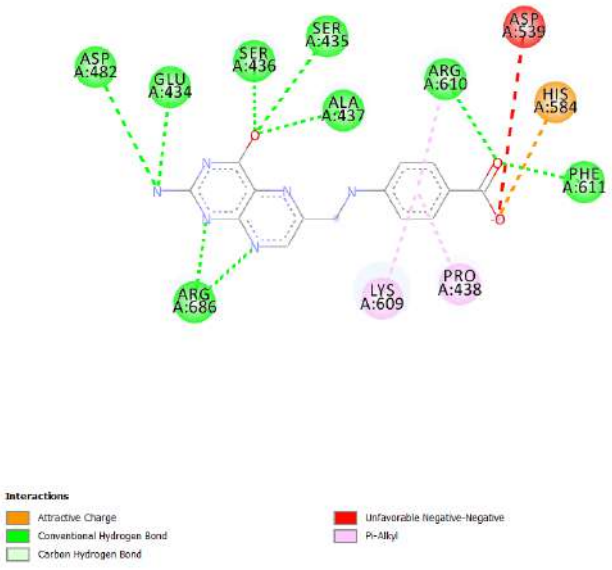
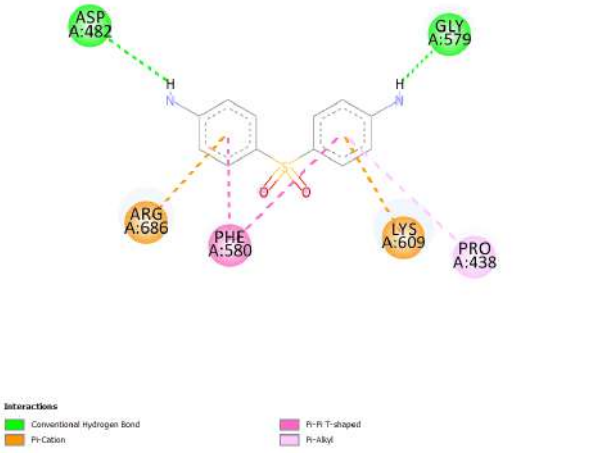
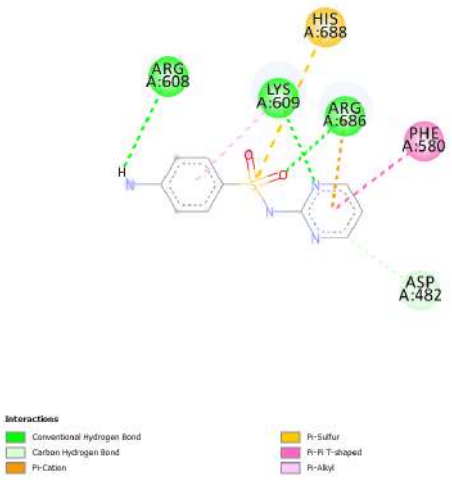
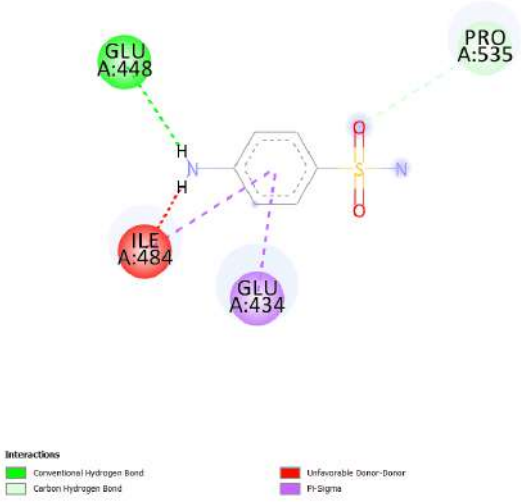
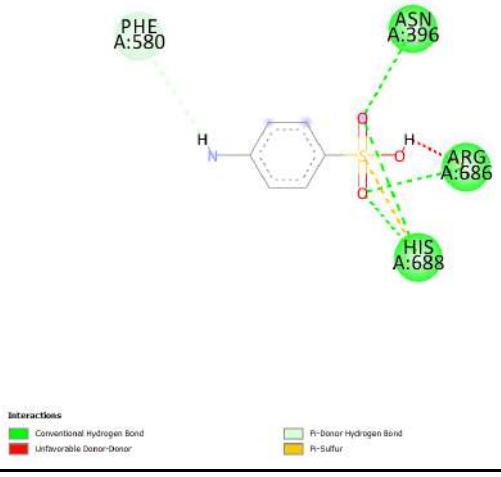
APPENDICES

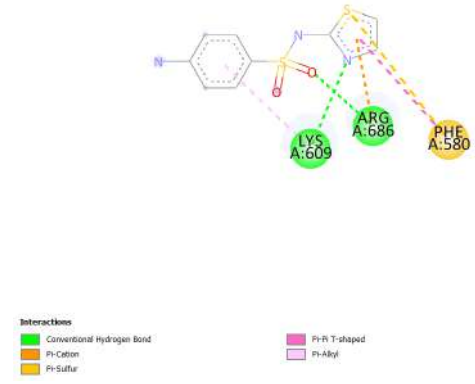
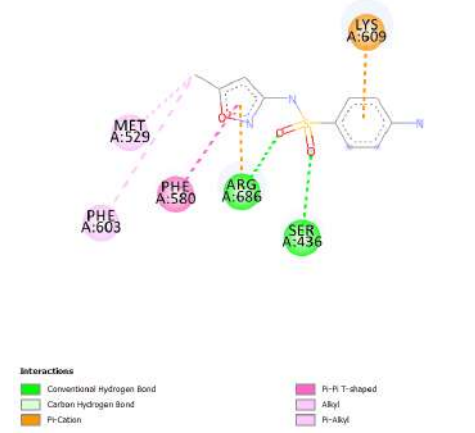
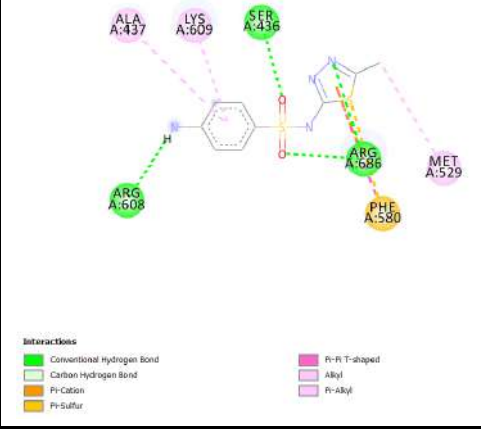
Appendix A- Summary table

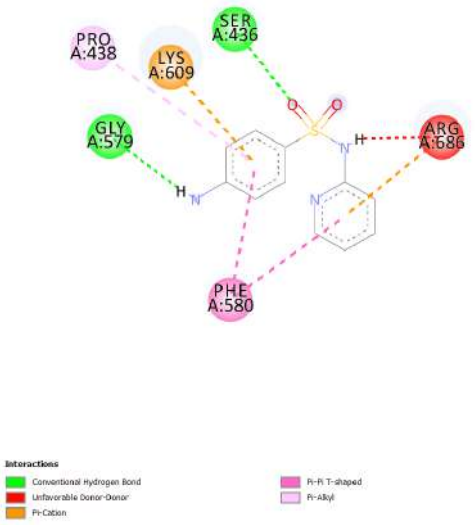
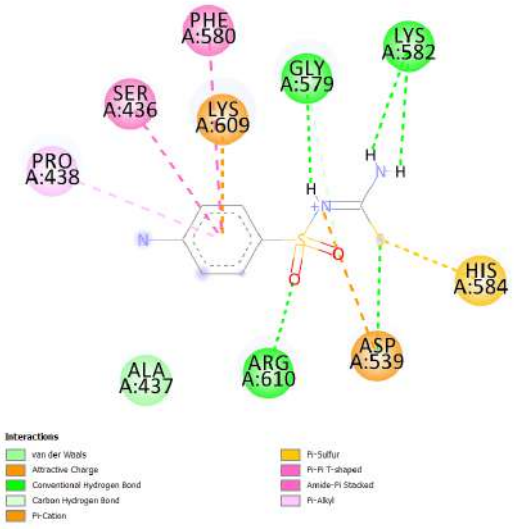
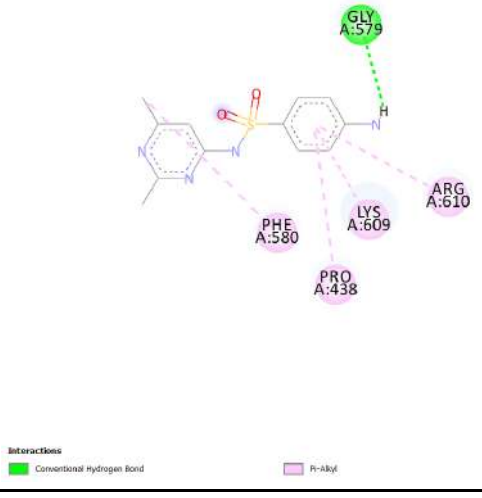
Table A: 2D diagram of twenty-two interactions of molecular docking in each PDB ID

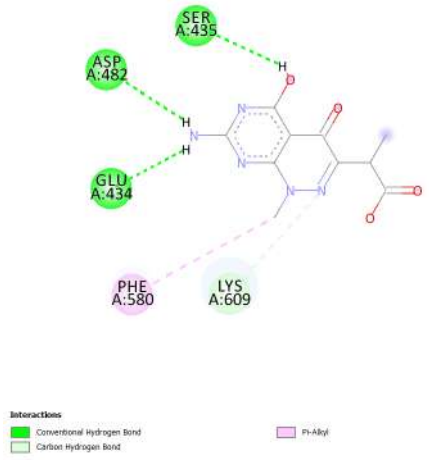
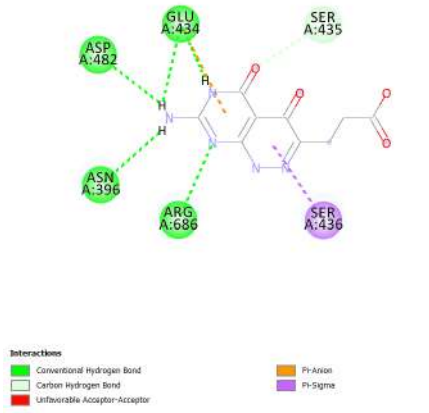
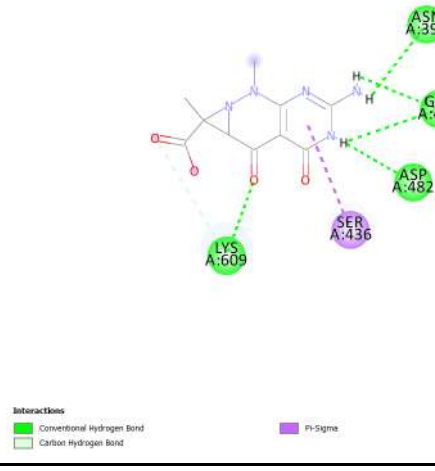
PDB ID: 6JWU (Wild-type)		
No.	**Ligand**	**Figure**
0.1	SDX-DHP	
0.2	STZ-DHP	

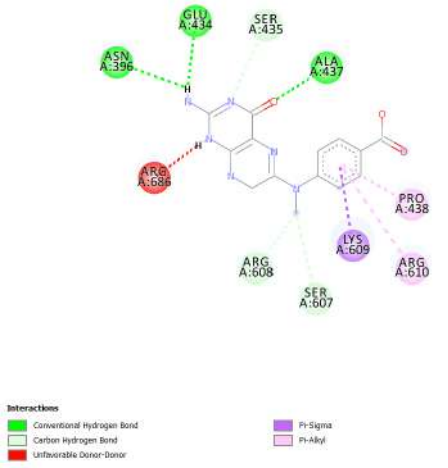
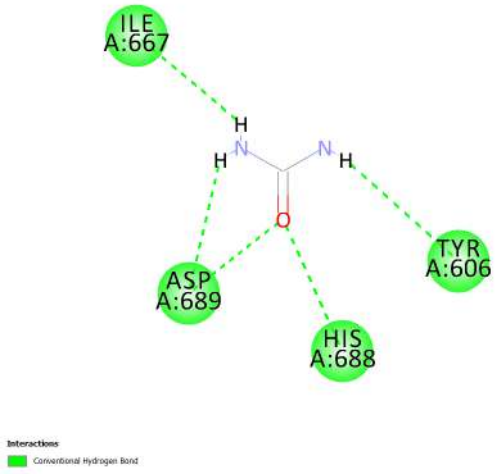
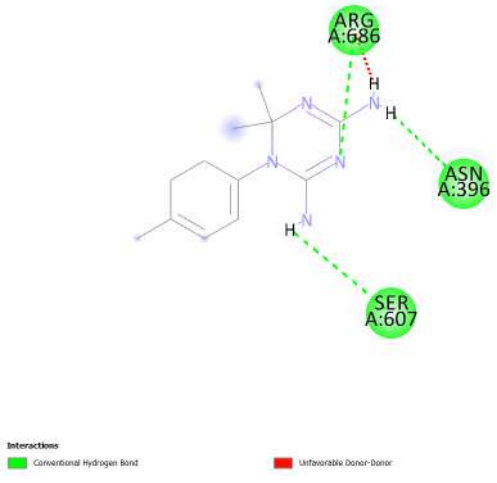
0.3	PTA	
1	4, 4'-Diaminodiphenylsulfone	

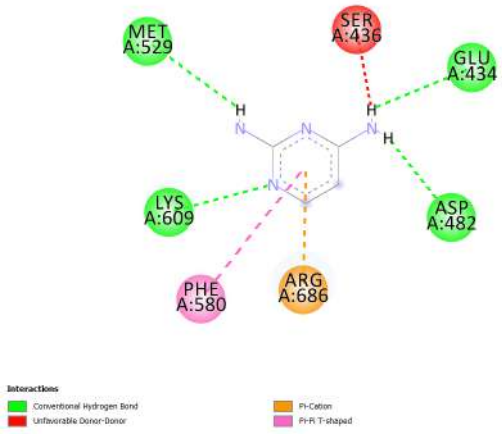
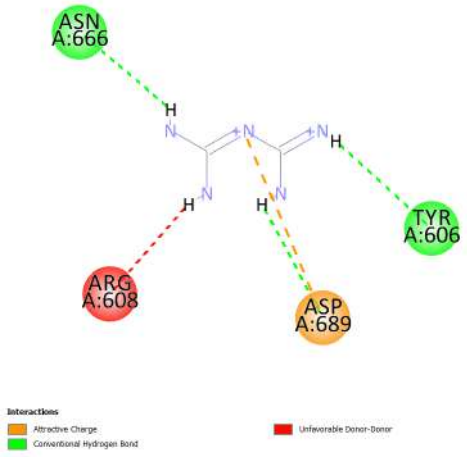
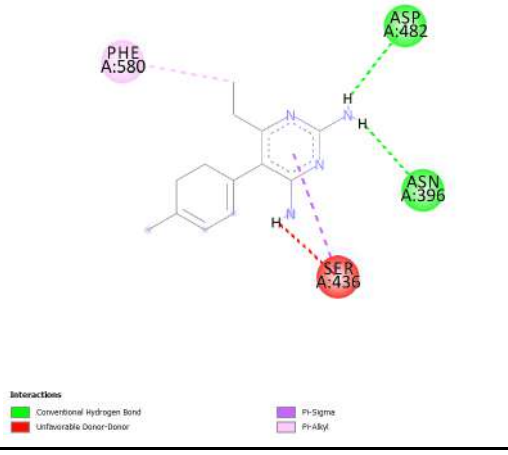
2	Sulfadiazine	
3	Sulfanilamide	
4	Sulfanilic acid	

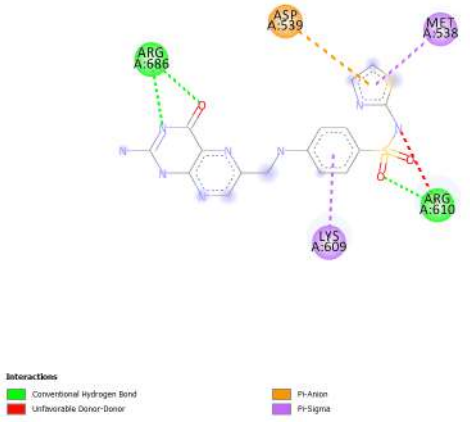
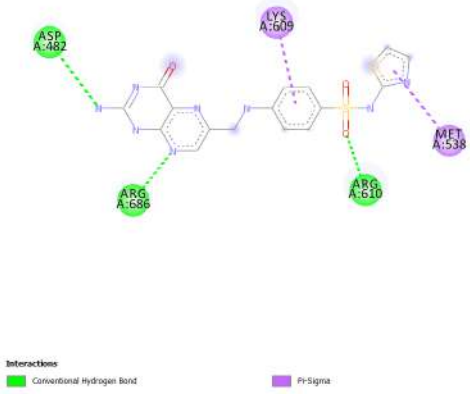
5	Sulfathiazole	
6	Sulfamethoxazole	
7	Sulfamethizole	

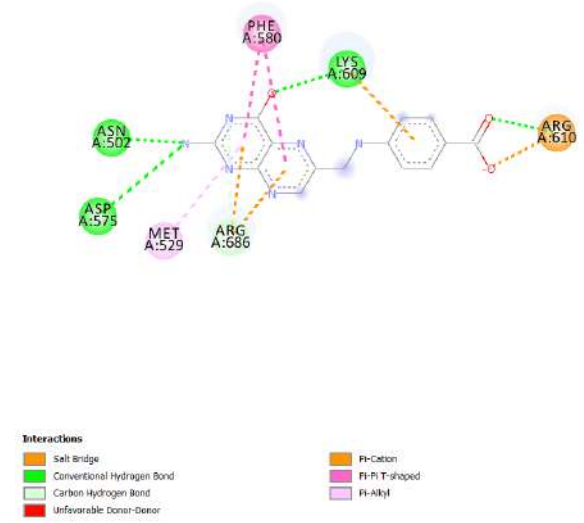
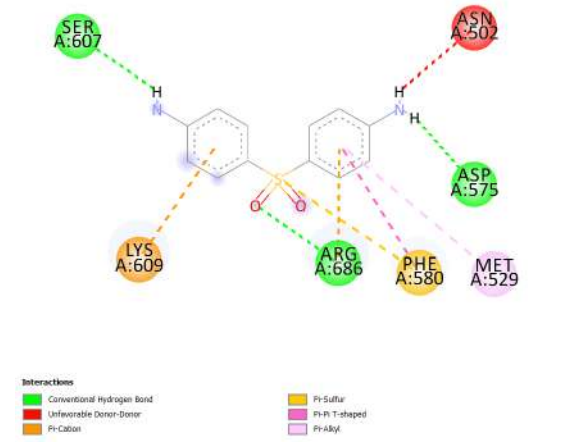
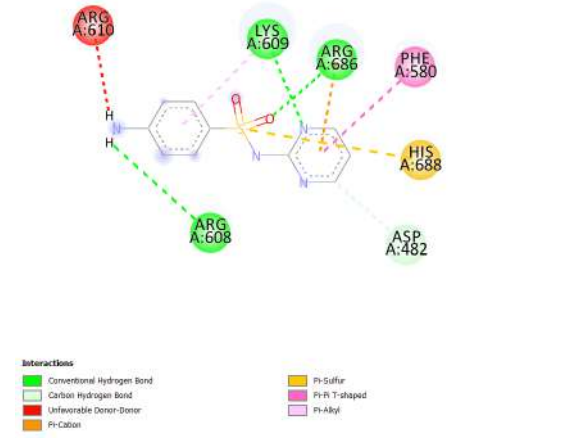
8	Sulfapyridine	
9	Sulfathiourea	
10	Sulfisomidine	

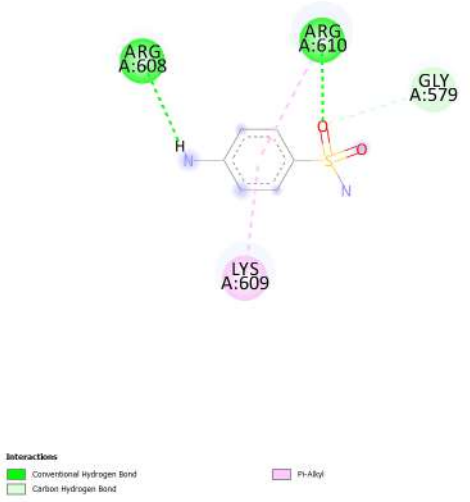
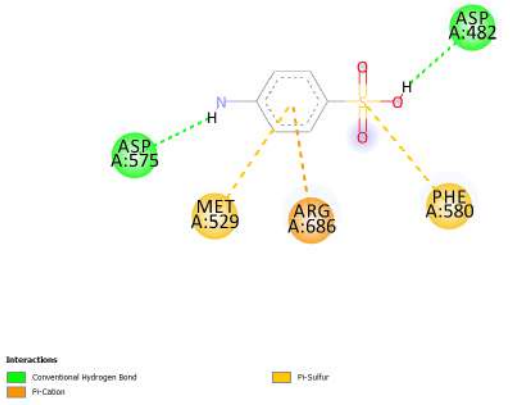
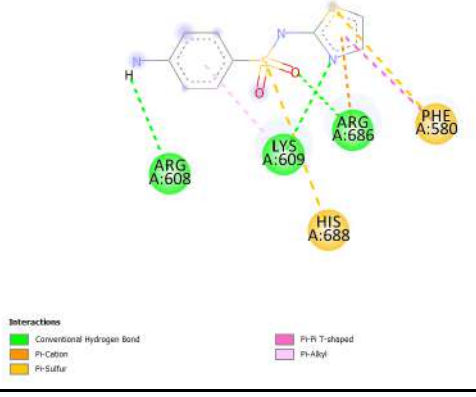
11	2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
12	3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
13	7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6 H-dione	

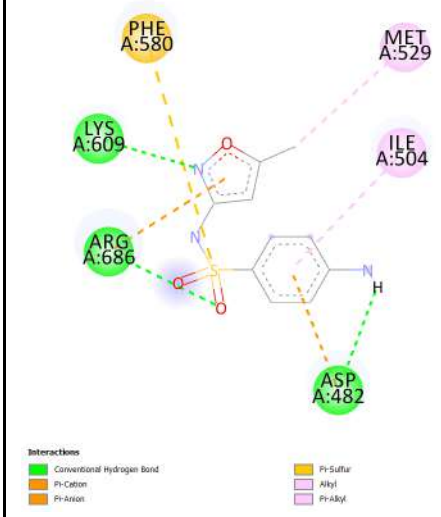
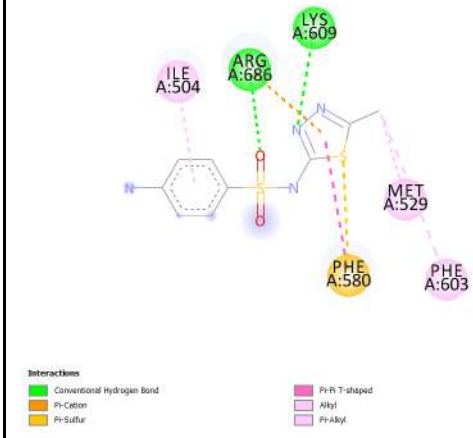
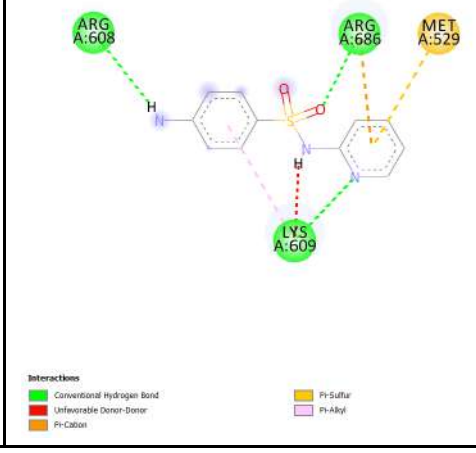
14	7,8-Dihydropteroic acid	
15	Urea	
16	Cycloguanil	

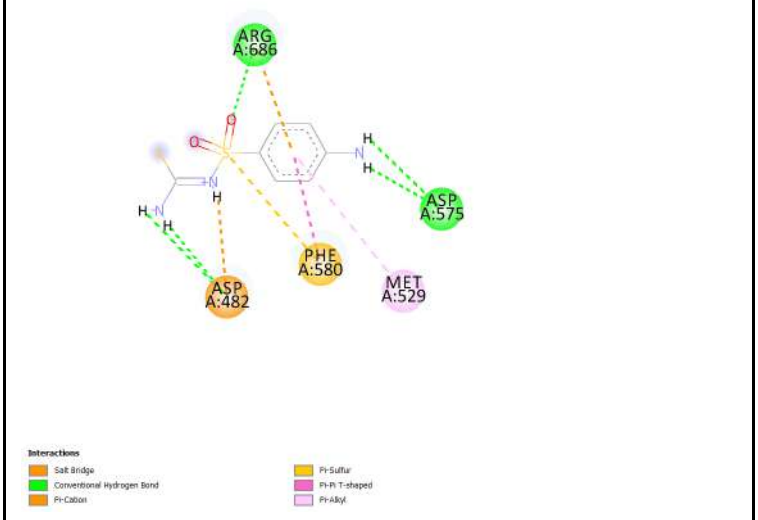
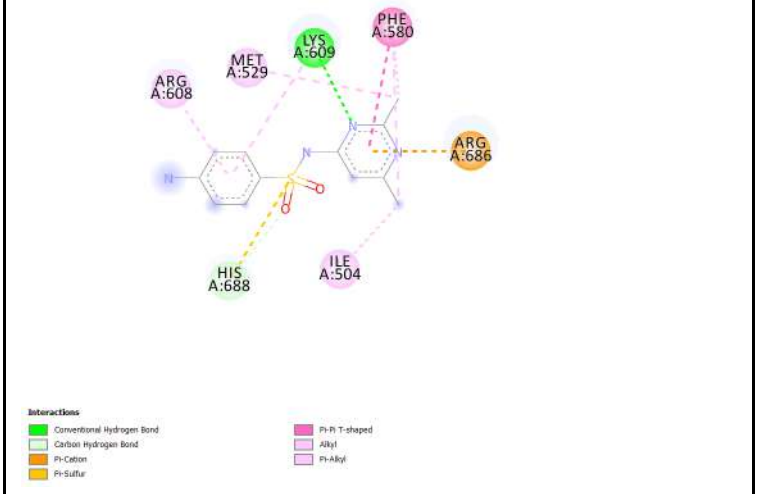
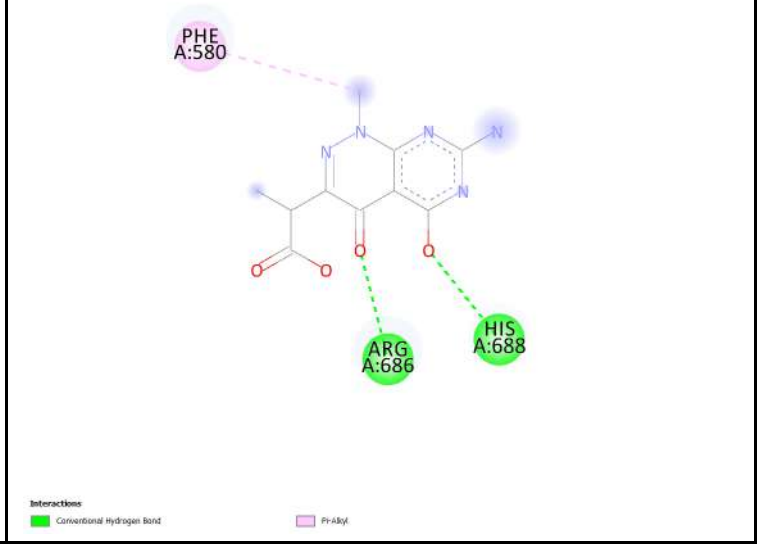
17	2,4-Diaminopyrimidine	
18	Biguanide	
19	Pyrimethamine	

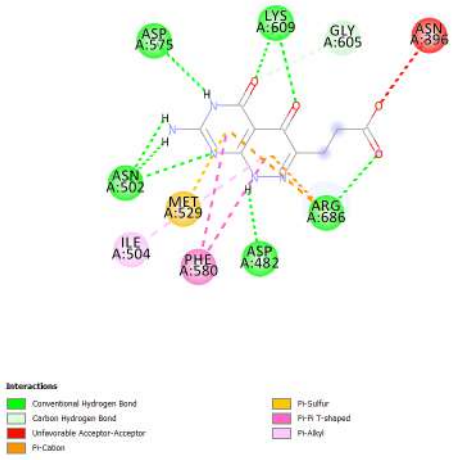
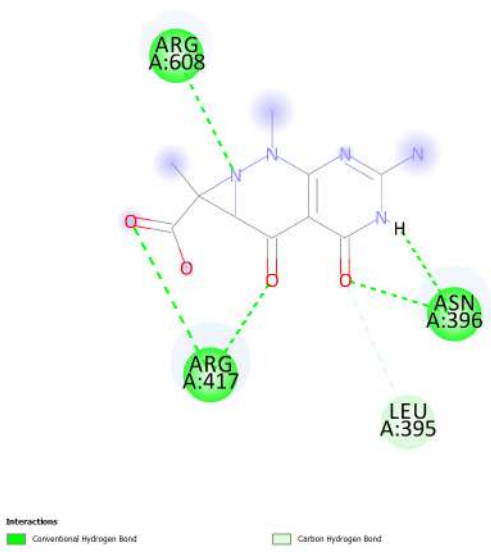
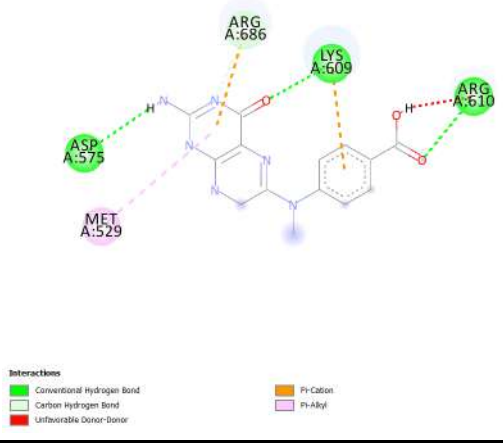
PDB ID : 6JWV (mutant)		
No.	**Ligand**	**Figure**
0.1	SDX-DHP	
0.2	STZ-DHP	

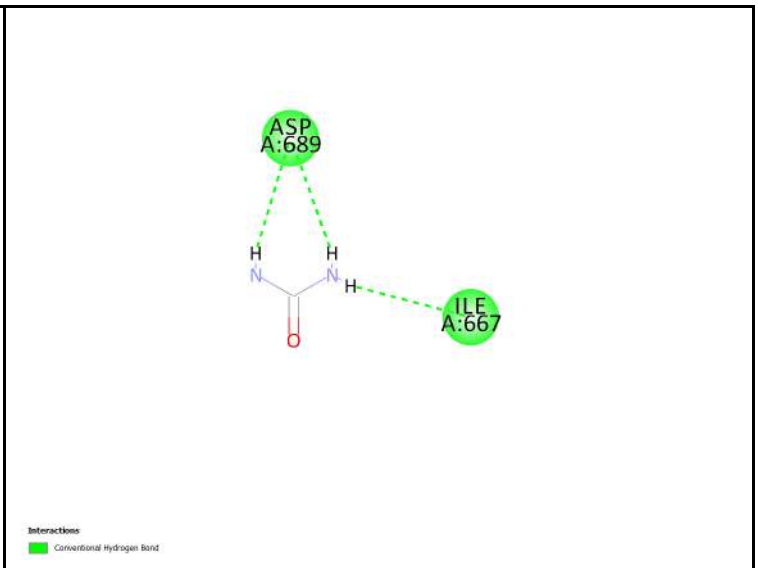
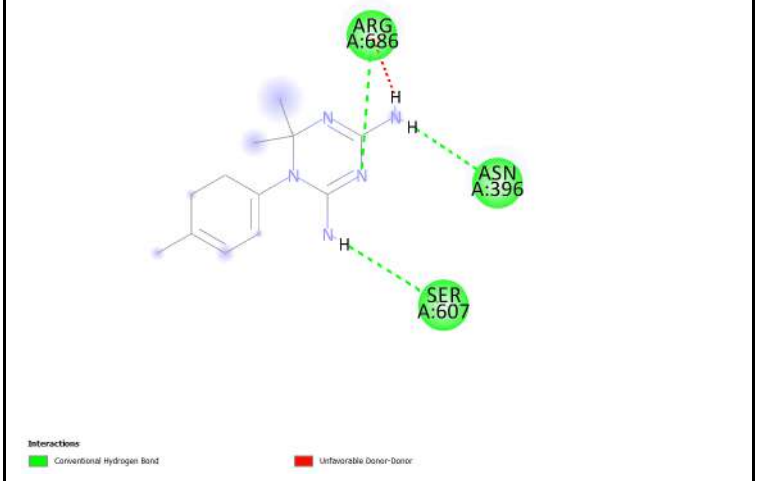
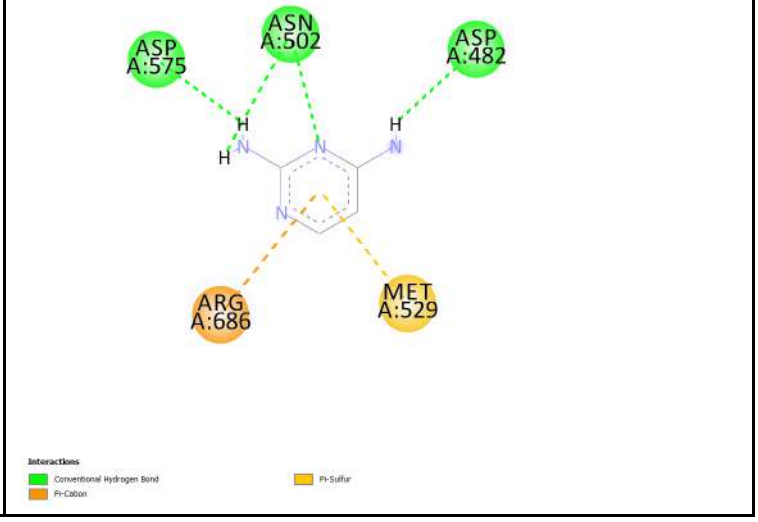
0.3	PTA	
1	4, 4'-Diaminodiphenylsulfone	
2	Sulfadiazine	

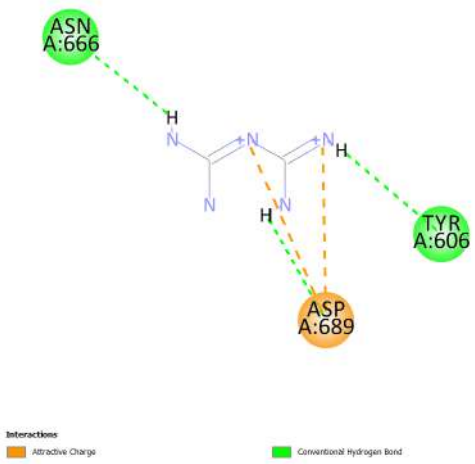
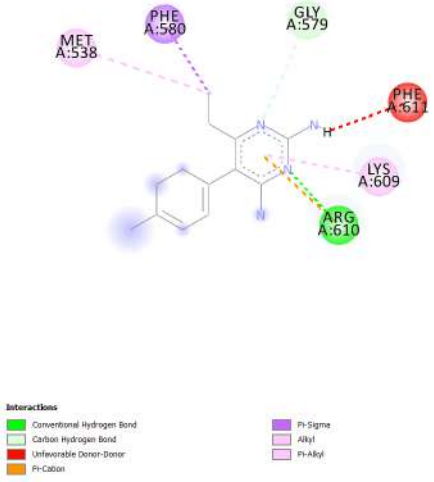
3	Sulfanilamide	
4	Sulfanilic acid	
5	Sulfathiazole	

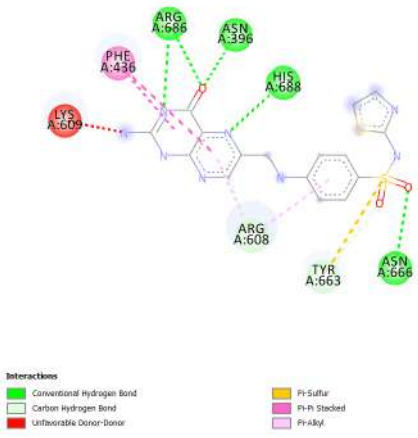
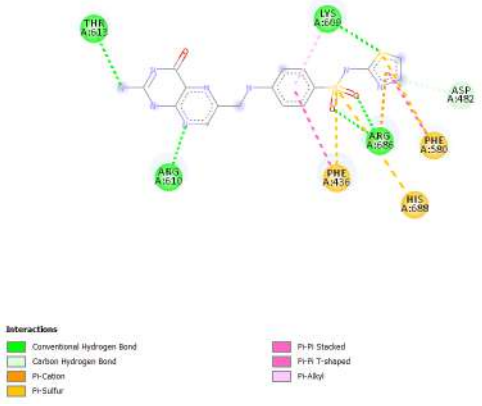
6	Sulfamethoxazole	
7	Sulfamethizole	
8	Sulfapyridine	

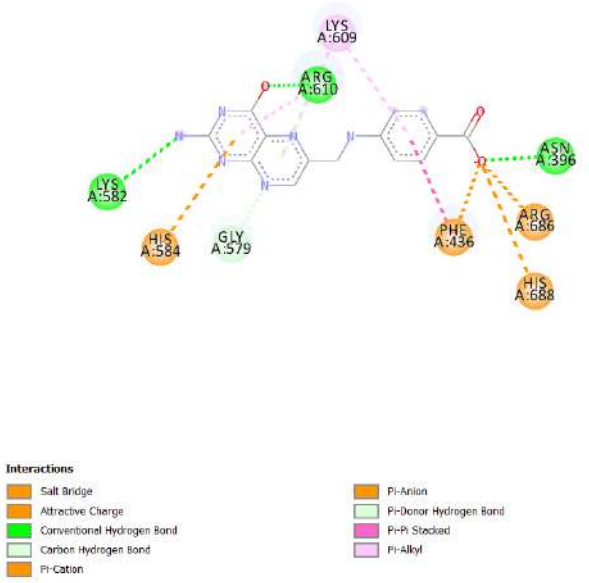
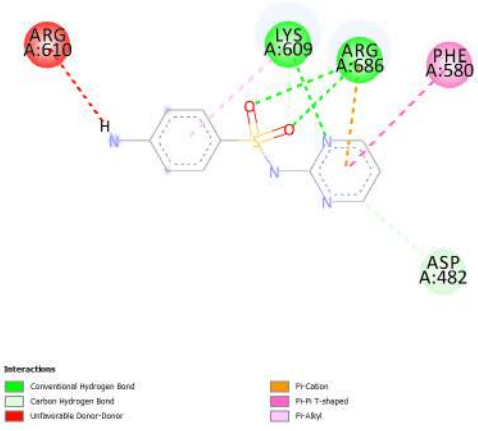
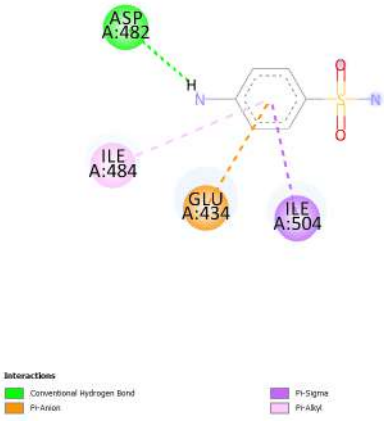
9	Sulfathiourea	
10	Sulfisomidine	
11	2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	

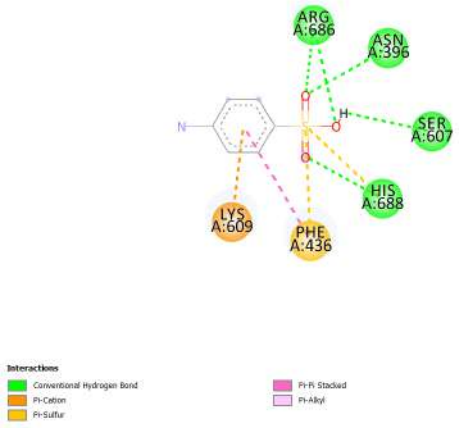
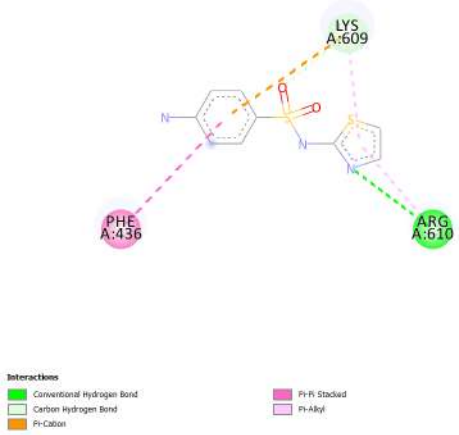
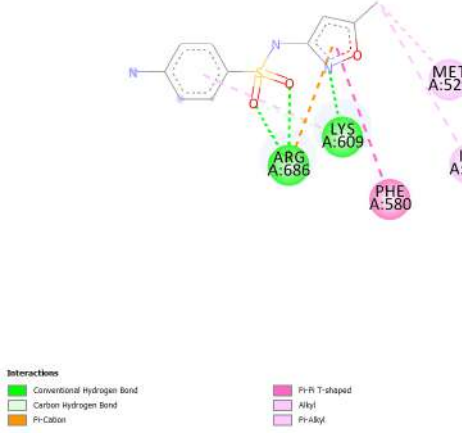
12	3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
13	7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6H-dione	
14	7,8-Dihydropteroic acid	

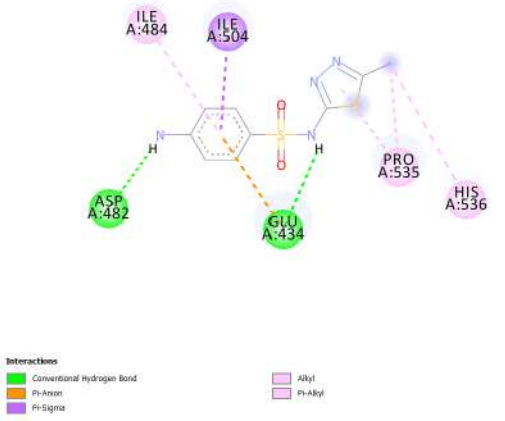
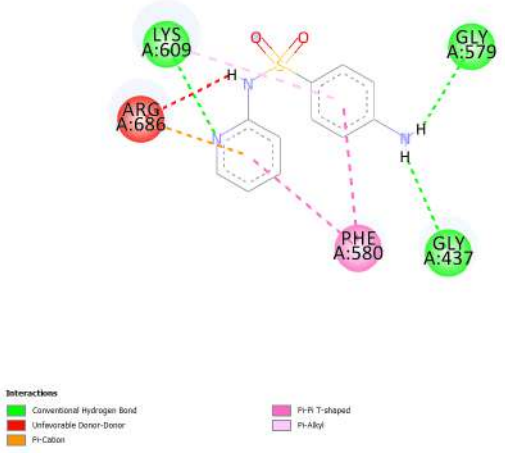
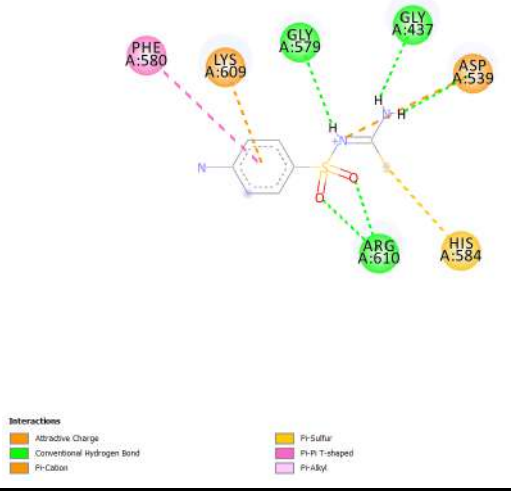
15	Urea	
16	Cycloguanil	
17	2,4-Diaminopyrimidine	

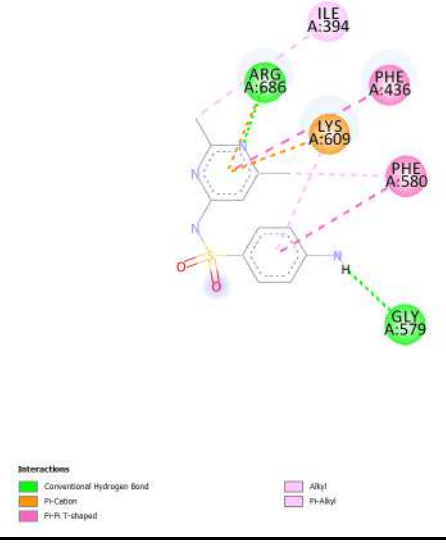
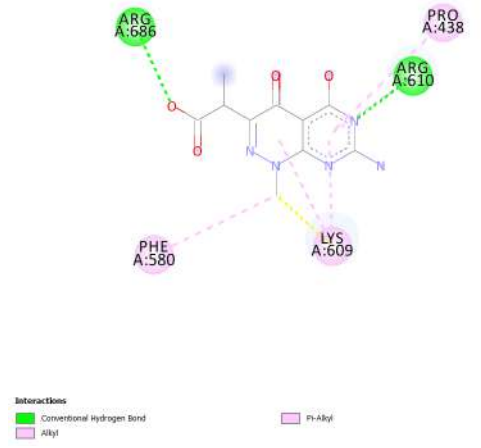
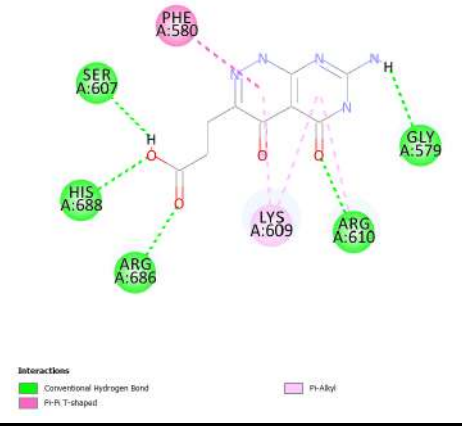
18	Biguanide	
19	Pyrimethamine	

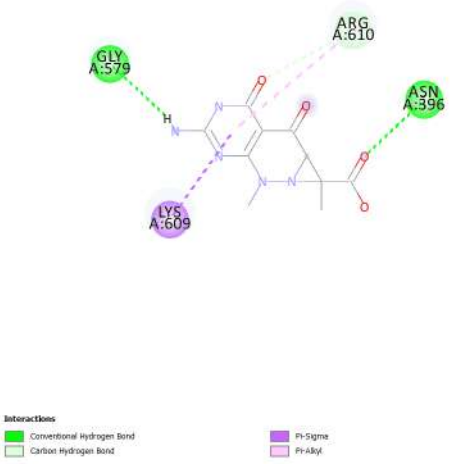
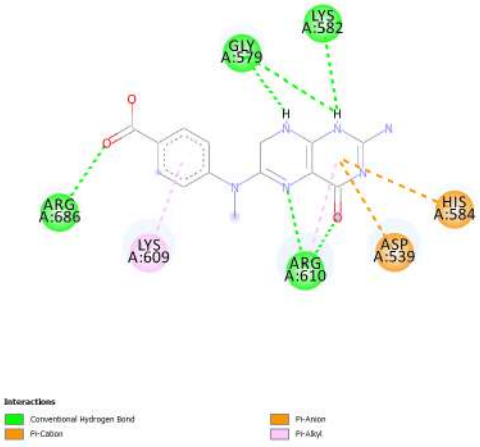
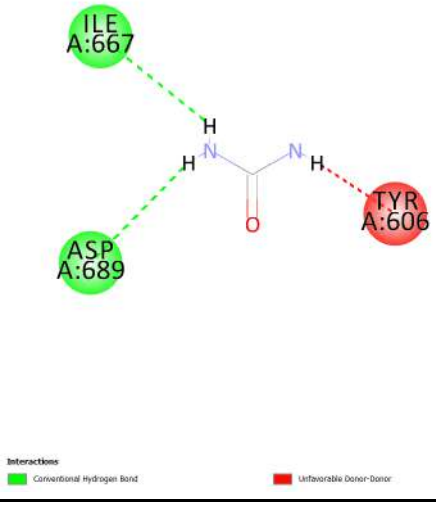
PDB ID : 6JWW (mutant)		
No.	**Ligand**	**Figure**
0.1	SDX-DHP	
0.2	STZ-DHP	

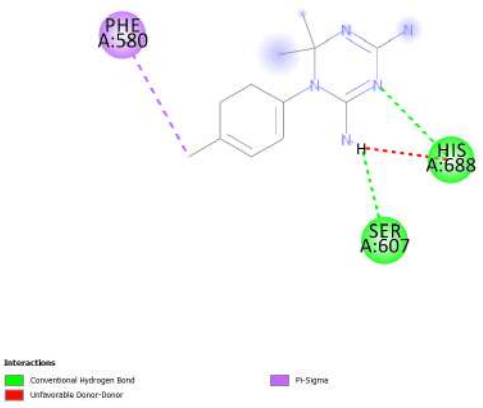
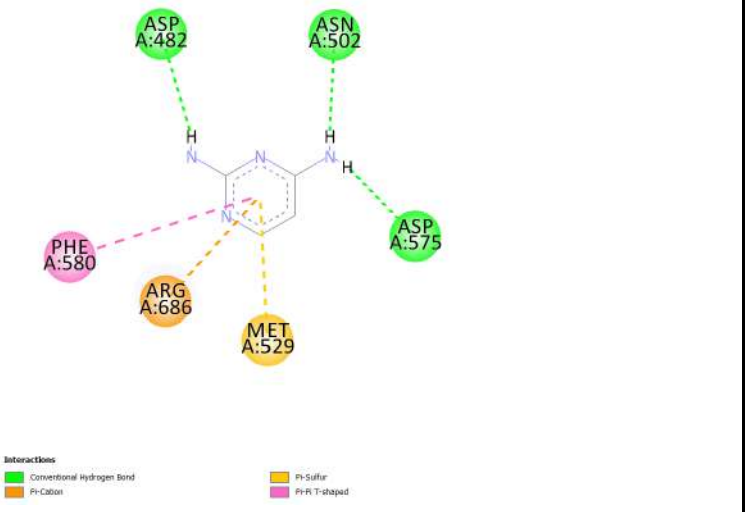
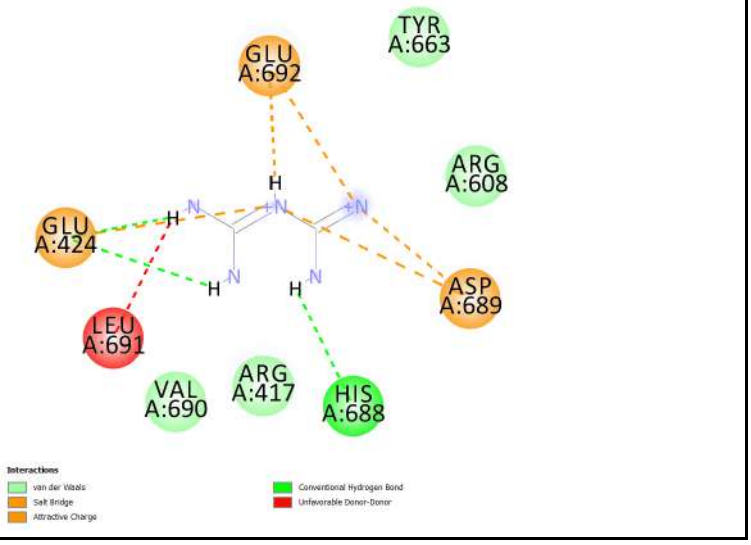
0.3	PTA	
2	Sulfadiazine	
3	Sulfanilamide	

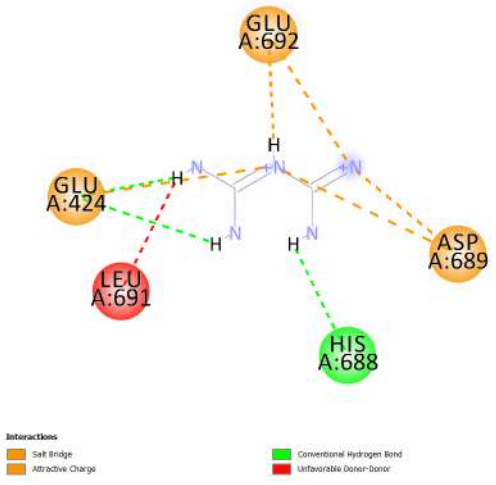
4	Sulfanilic acid	
5	Sulfathiazole	
6	Sulfamethoxazole	

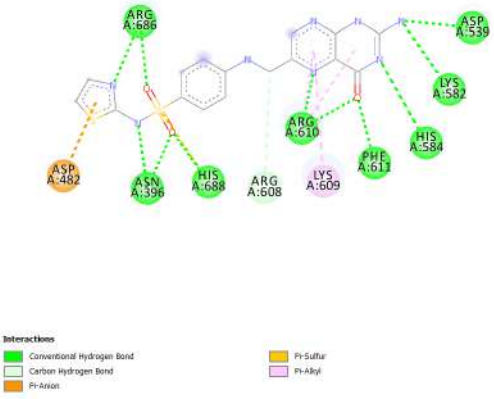
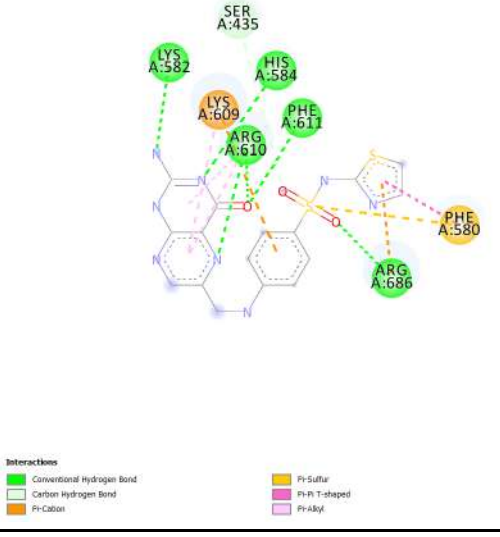
7	Sulfamethizole	
8	Sulfapyridine	
9	Sulfathiourea	

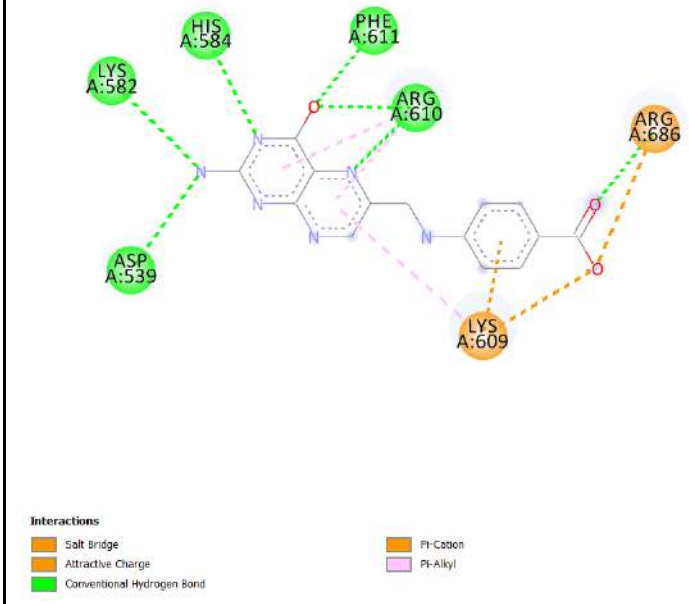
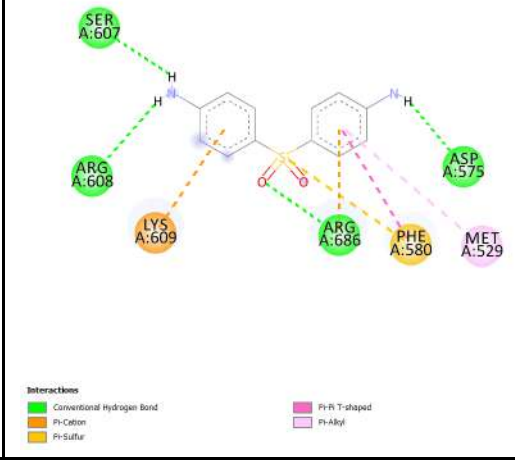
10	Sulfisomidine	
11	2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
12	3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	

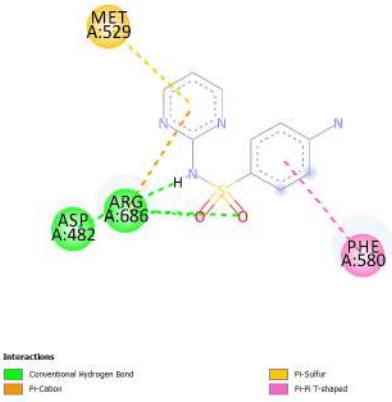
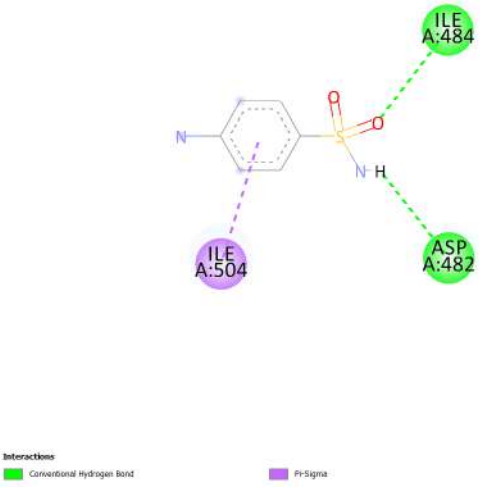
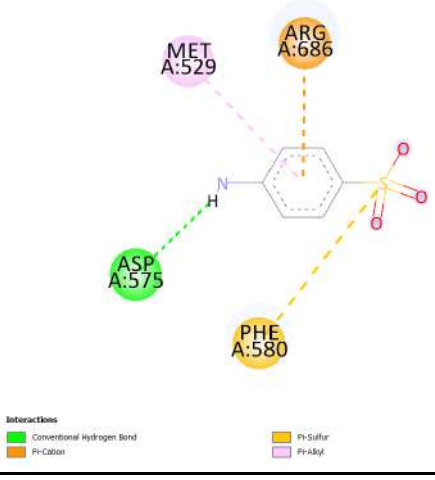
13	7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6H-dione	
14	7,8-Dihydropteroic acid	
15	Urea	

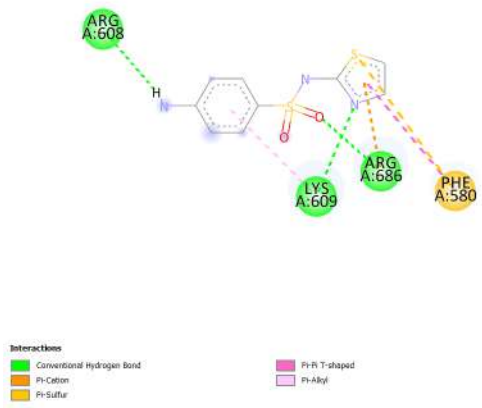
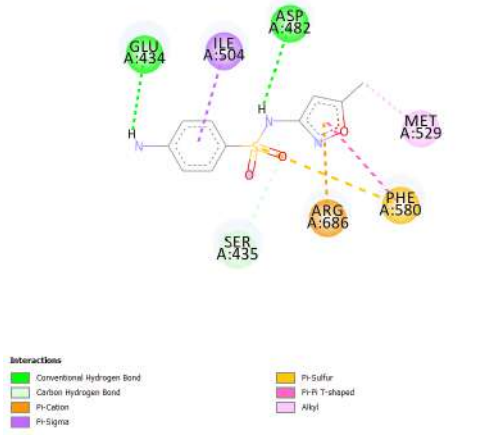
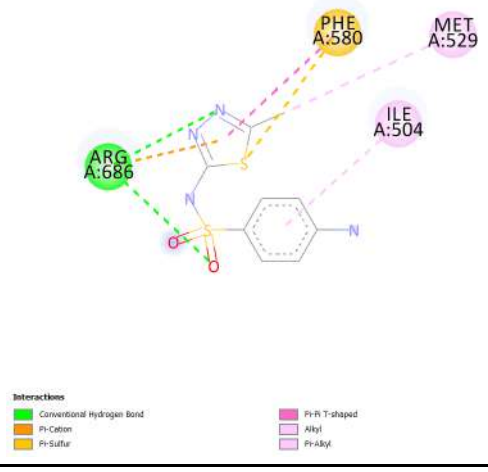
16	Cycloguanil	
17	2,4-Diaminopyrimidine	
18	Biguanide	

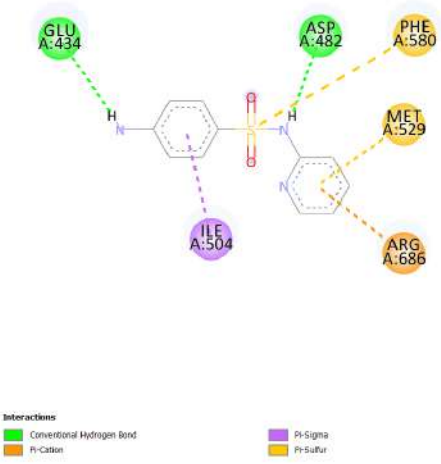
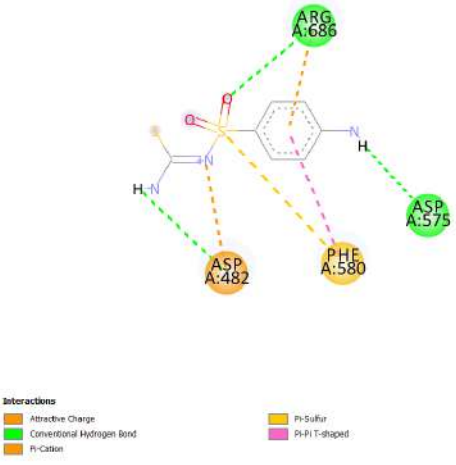
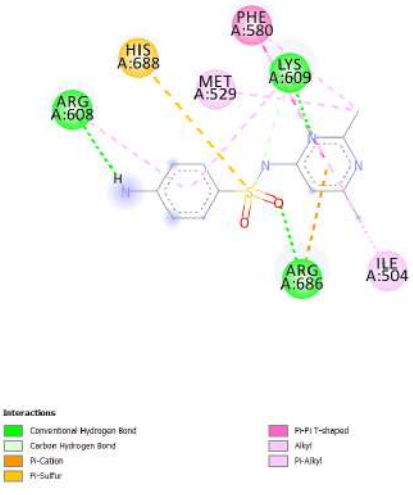
19	Pyrimethamine	

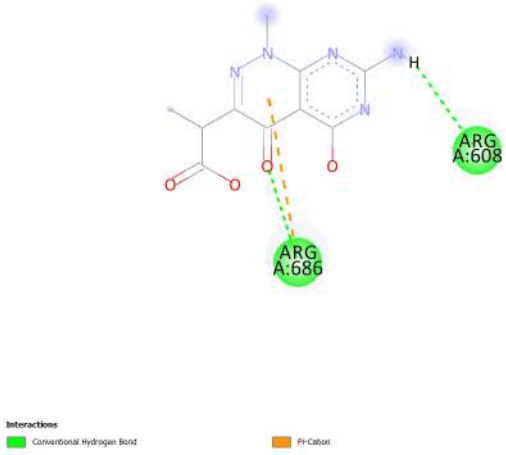
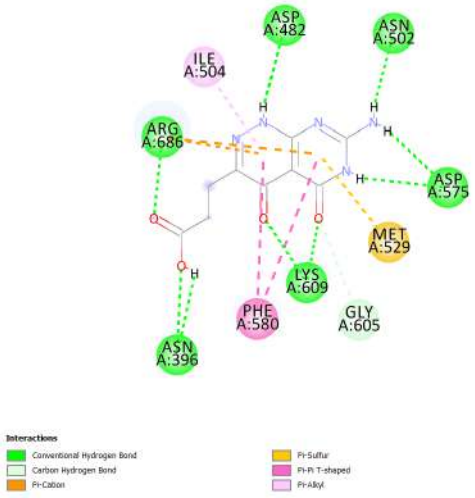
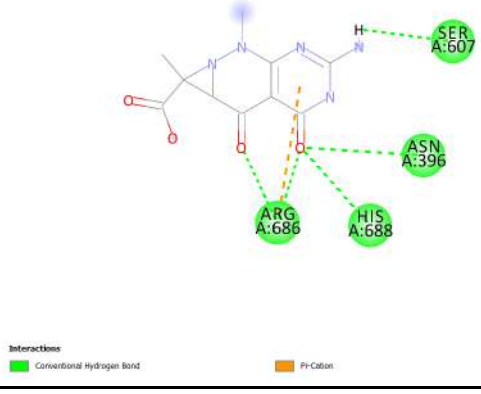
PDB ID : 6JWZ (mutant)		
No.	**Ligand**	**Figure**
0.1	SDX-DHP	
0.2	STZ-DHP	

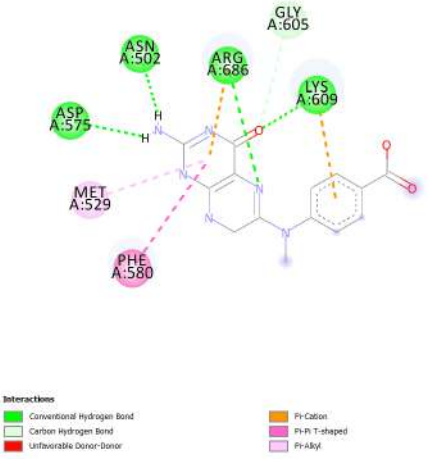
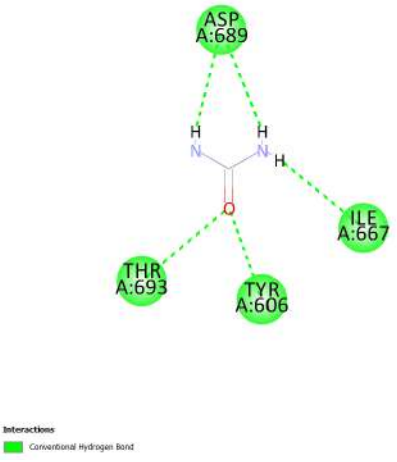
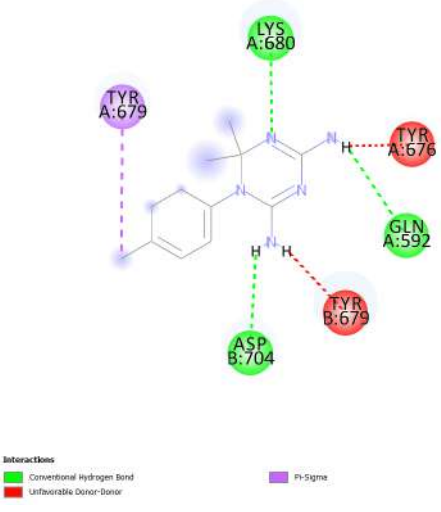
0.3	PTA	
1	4, 4'-Diaminodiphenylsulfone	

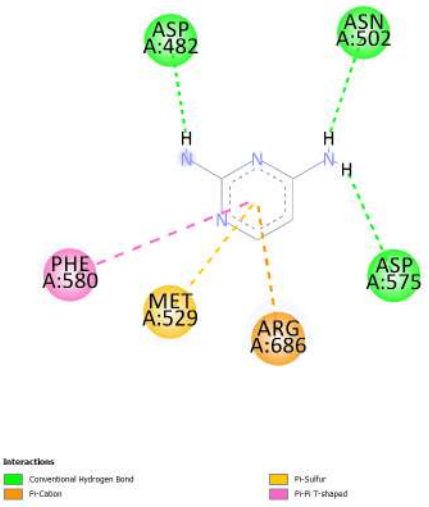
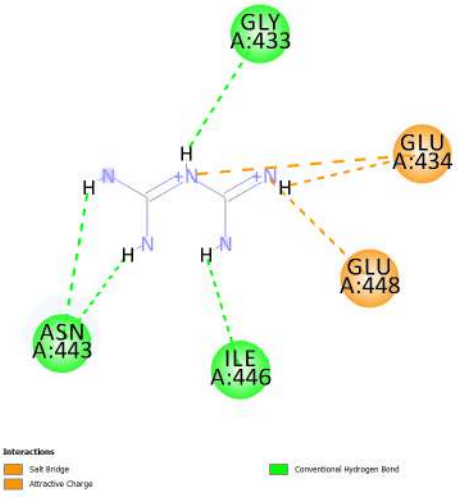
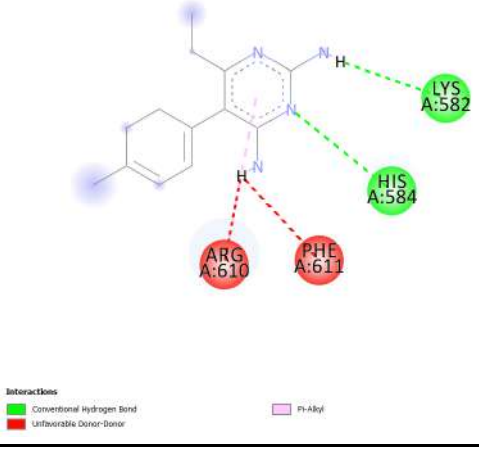
2	Sulfadiazine	
3	Sulfanilamide	
4	Sulfanilic acid	

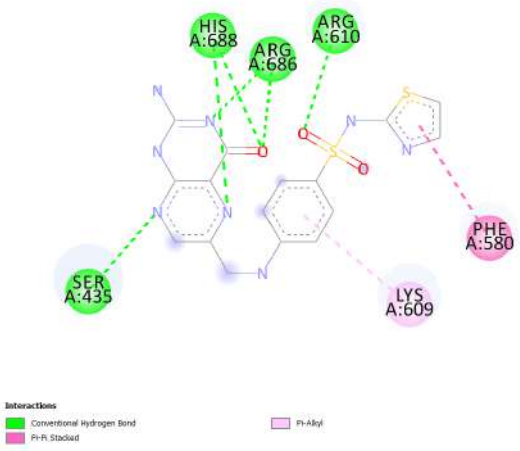
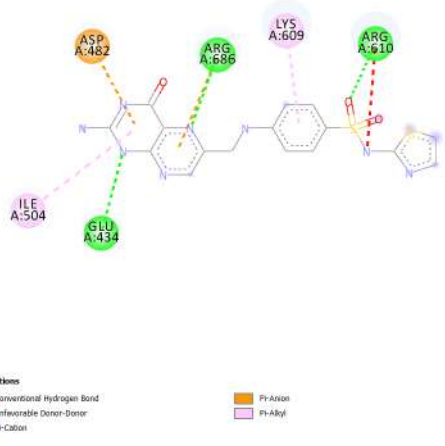
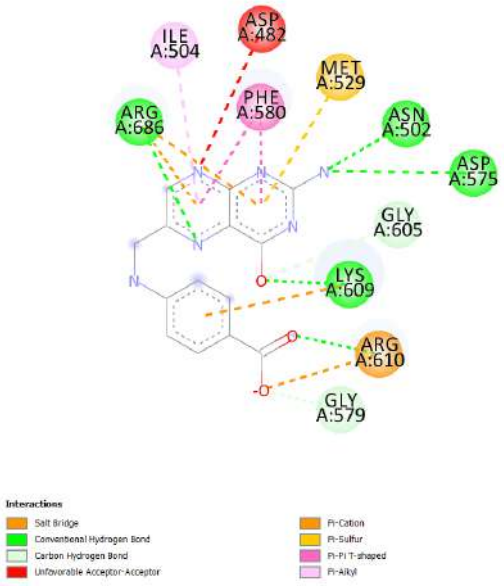
5	Sulfathiazole	
6	Sulfamethoxazole	
7	Sulfamethizole	

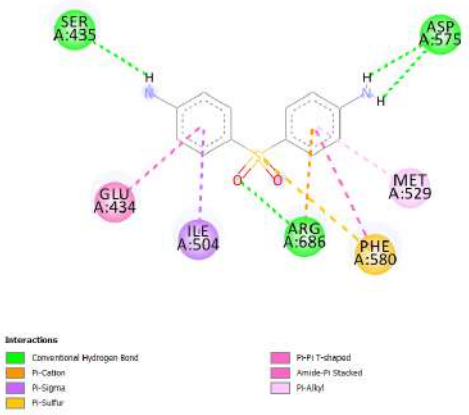
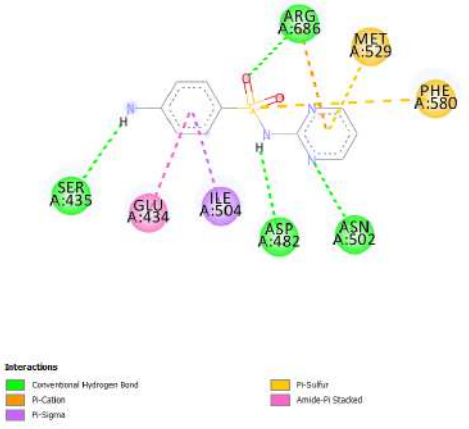
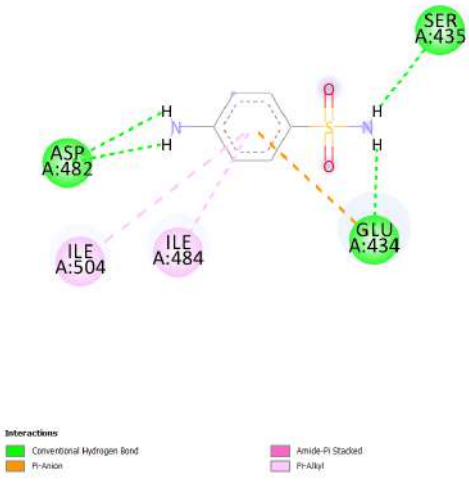
8	Sulfapyridine	
9	Sulfathiourea	
10	Sulfisomidine	

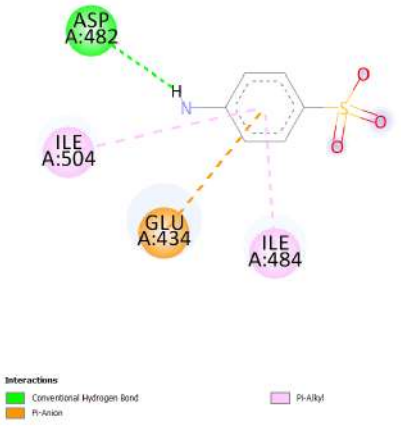
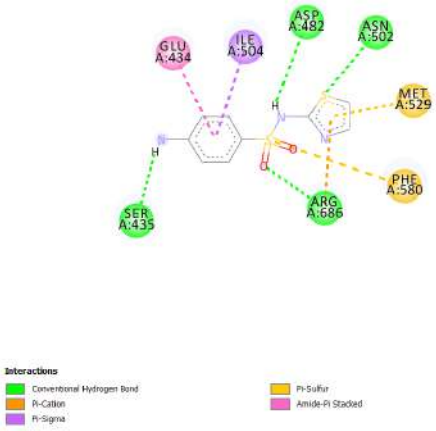
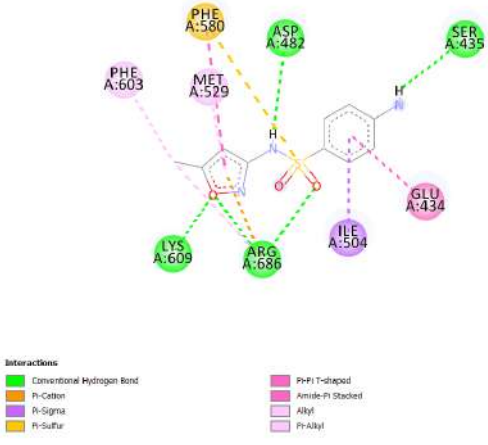
11	2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
12	3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
13	7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6H-dione	

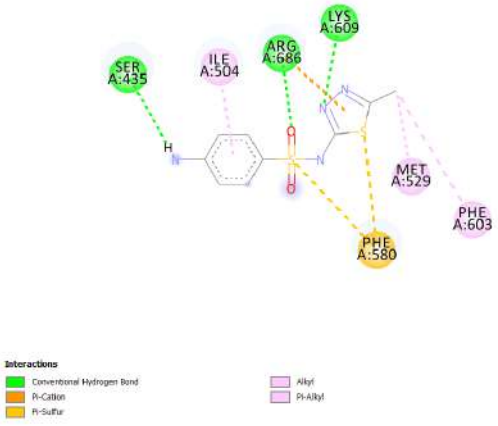
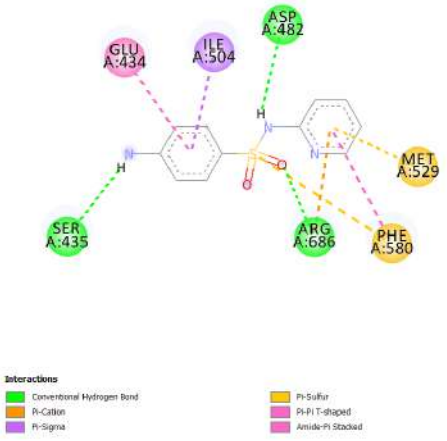
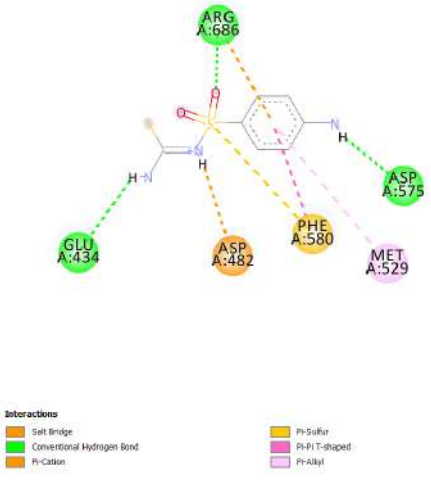
14	7,8-Dihydropteroic acid	
15	Urea	
16	Cycloguanil	

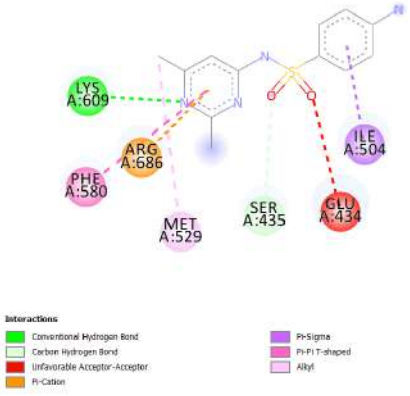
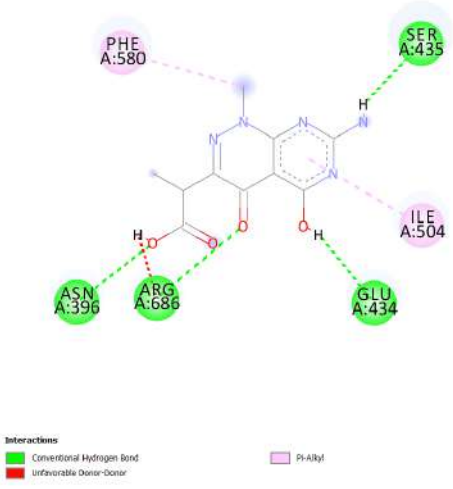
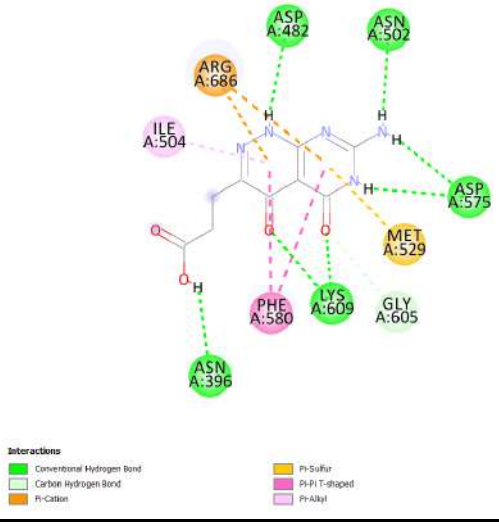
17	2,4-Diaminopyrimidine	
18	Biguanide	
19	Pyrimethamine	

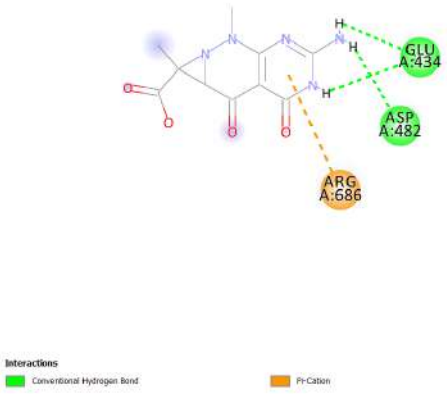
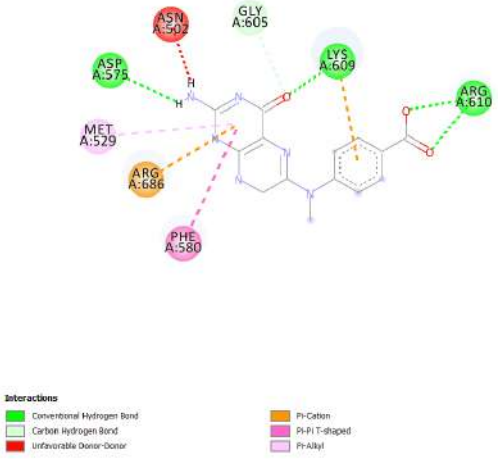
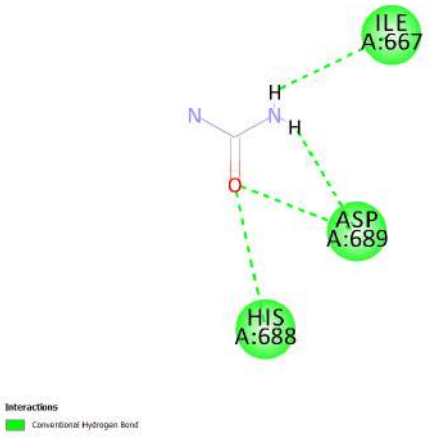
PDB ID : 6KCL (mutant)		
No.	**Ligand**	**Figure**
0.1	SDX-DHP	
0.2	STZ-DHP	
0.3	PTA	

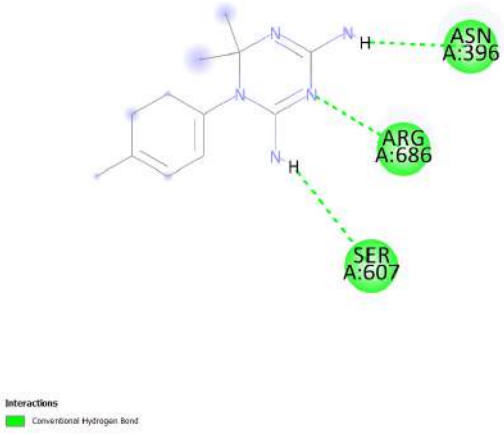
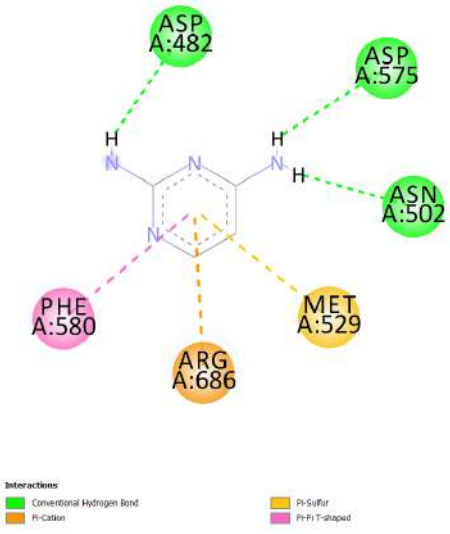
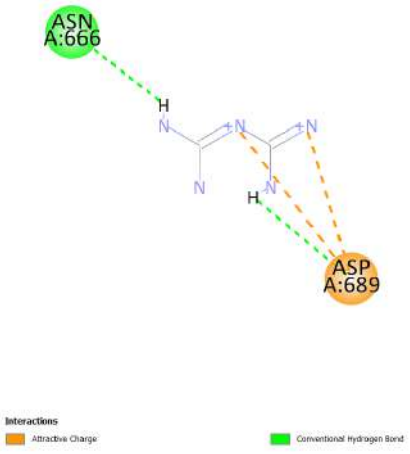
1	4, 4'-Diaminodiphenylsulfone	
2	Sulfadiazine	
3	Sulfanilamide	

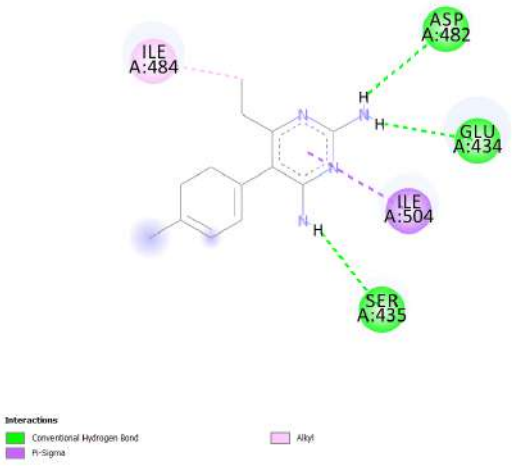
4	Sulfanilic acid	
5	Sulfathiazole	
6	Sulfamethoxazole	

7	Sulfamethizole	
8	Sulfapyridine	
9	Sulfathiourea	

10	Sulfisomidine	
11	2-(7-Amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	
12	3-(7-Amino-4,5-dioxo-1,4,5,6-tetrahydropyrimido[4,5-c]pyridazin-3-yl)propanoic acid	

13	7-amino-3-1-carboxyethyl-1-methyl-pyrimido-4-5-c-pyridazine-4-51H-6H-dione	
14	7,8-Dihydropteroic acid	
15	Urea	

16	Cycloguanil	
17	2,4-Diaminopyrimidine	
18	Biguanide	

19	Pyrimethamine	

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