Applied Chemistry Project

Project title	Molecular docking calculations of *Helicobacter pylori* and its inhibitors

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Program	Bachelor of Science in Applied Chemistry		
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Faculty of Science, Chulalongkorn University

Molecular Docking Calculations of *Helicobacter Pylori* and Its Inhibitors

by

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In Partial Fulfillment for the Degree of Bachelor of Science

Program in Applied Chemistry (International Program)

Department of Chemistry, Faculty of Science

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Project Molecular docking calculations of *Helicobacter pylori* and its inhibitors

By Mr. Chisanupong Kunmas and Mr. Panuwat Viriyaparadon

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Abstract

H. pylori is a bacterium usually found in the stomach. *H. pylori* infection sometimes causes gastritis or ulcers of the stomach and has been link to a wide range of other diseases. Purine nucleoside phosphorylase (PNP) is an essential enzyme in the purine salvage pathway of *H. pylori*. It is thought that binding of phosphate, one of the substrates, induces a conformational change in the active site of the enzyme. It is thus interesting to investigate whether the presence of phosphate in the active site will affect the binding mode of inhibitors. Totally 20 inhibitors were computationally docked into the *H. pylori* PNP structures (HpPNP) with and without phosphate and the docked configuration of each ligand to the two HpPNP structures were comparatively analyzed. The results showed that most ligands bind to HpPNP differently when there is phosphate in the active site.

Keywords: *H. pylori*, purine nucleoside phosphorylase (PNP), molecular docking, phosphate, inhibitor

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Chapter 1

Introduction

1.1 Introduction to the research problem and significance

Helicobacter Pylori (*H. pylori*), previously known as *Campylobacter pylori*, is a gram negative, microaerophilic, spiral bacterium usually found in the stomach. Its helical shape is thought to have evolved in order to penetrate the mucoid lining of the stomach and establish infection. *H. pylori* has been associated with the mucosa associated lymphoid tissue in the stomach, esophagus, colon, rectum, or tissues around the eye, and of lymphoid tissue in the stomach.

H. pylori infection usually has no symptoms but sometimes causes gastritis or ulcers of the stomach or first part of the small intestine. The infection is also associated with the development of certain cancers occurring in less than 20% of cases. Many investigators suggested that *H. pylori* causes a wide range of other diseases, for example idiopathic thrombocytopenic purpura, Alzheimer's disease, coronary artery disease, Parkinson's disease etc. The bacterial infection has also been proposed to have protective effects for its hosts against infections by other pathogens. In 2015, it was estimated that over 50% of the world's population had *H. pylori* in their upper gastrointestinal tracts with this infection being more common in developing countries [4]. In recent decades, however, the prevalence of *H. pylori* colonization of the gastrointestinal tract has declined in many countries.

H. pylori does not have ability to synthesize purine de novo so it must rely on the purine salvage pathway to synthesize purines as indispensable building blocks for its DNA and RNA synthesis. Purine nucleoside phosphorylase (PNP) is an essential enzyme in the purine salvage pathway. It catalyzes the conversion of inosine and guanosine to hypoxanthine and guanine, respectively. The reaction that PNP catalyzes is the cleavage of the glycosidic bond of purine (2'-deoxy) nucleosides, leading to free base and (2'-deoxy) ribose-1-phosphate as products [3]. Phosphate is one of the substrates. It is thought that binding of phosphate induces a conformational change in the active site of the enzyme, and this is a necessary initial step in the catalysis. Therefore, it is interesting to investigate whether the presence of phosphate in the active site will affect the binding mode of inhibitors.

In order to find the inhibitor of H. pylori, researchers use the molecular docking method. Molecular docking is the study of how two or more molecular structure fit together. In simple definition, docking is a molecular modeling technique that used to predict how a protein (enzyme) interacts with small molecules (ligands). Molecular docking is one of the largely acclaimed

structure-based approaches, widely use for the study of molecular recognition, which aims to predict the binding mode and binding affinity of a complex formed by two or more constituent molecules with known structures.

1.2 Research objectives

The research objective is to find out whether phosphate will affect the binding configuration between *H. pylori* PNP (HpPNP) and its inhibitors by using molecular docking technique.

1.3 Literature search

Montgomery et al. [1] developed inhibitors for purine nucleoside phosphorylase in T-cell, which take a role on inhibiting the catalyze of enzyme in the active site. In this article there are three main groups of inhibitors that used docking in the binding site of PNP, determined by X-ray crystallography.

Bošnjaković et al. [2] investigated the role of phosphate binding in PNP of *H. pylori* by soaking the crystals of apo form of the enzyme in increasing concentrations of phosphate. They found that the binding of phosphate to the active site of PNP from *H. pylori* does not cause the conformational change of the active site. However, many external factors, which influence phosphate binding, could not be controlled in this study. Therefore, it is still not certain about the exact role of phosphate binding.

Narczyk et al. [3] extensively investigated the structure of the apo forms of hexameric PNP from *H. pylori* and its complexes with phosphate (Pi) and an inhibitor, formycin A (FA) by using X-ray crystallography, molecular docking calculation, and molecular dynamics simulation.

Chapter 2

Experiment

2.1 List of software

1. BIOVIA Discovery Studio Visualizer 2020, denoted as DS Viewer
2. HyperChem 8.0.10
3. AutoDockTools 1.5.4, denoted as ADT
4. OpenBabel 2.4.0
5. VEGA ZZ 3.2.1, denoted as Vega

2.2 Experiment procedures

1. Preparation of HpPNP structure
   - Downloaded the X-ray crystallographic structure of HpPNP from the Protein Data Bank (PDB)

     Model code: 6F4X [3] for HpPNP with PO_4 as substrate

     Model code: 6F4W [3] for HpPNP without PO_4 as substrate
   - Used DS Viewer to remove water and non-related molecules from the X-ray structures
   - Used ADT to add polar hydrogen atoms into structures and to assign all necessary parameters for the docking calculations
2. Preparation of ligand model
   - Drew three-dimensional structure of each ligand using HyperChem
   - Lists of used ligands from Montgomery, J.A. and Secrist, J.A. [1].
   - Defined as 3 groups of ligands:

1st group: 9-R-9-deazaguanine (see in figure 1), R group see in Table 1.

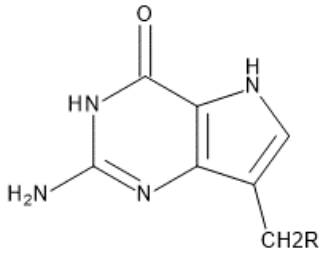

Figure 1. 9-R-9-deazaguanine

Table 1. 1st R's group and IC50

Number	Name	IC_{50}
21	3-Chlorophenyl	20 ± 7
23	4-Iodophenyl	23
24	3-Fluorophenyl	24
27	Pyridin-3-yl	25 ± 2.8
28	3-Trifluoromethylphe	36 ± 12
30	3-Methylphenyl	57
31	3-Hydroxypheny	70 ± 20
33	2-Furanyl	83
37	4-Hydroxyphenyl	260
38	2-Hydroxyphenyl	270

2nd group: 9-R-9-deazaguanine with cycloaliphatic substituents at the 9-position (see in figure 2), R group see in table 2.

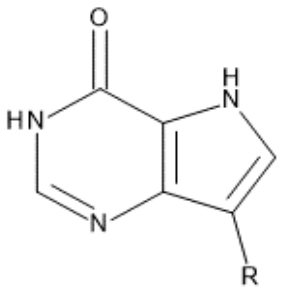

Figure 2. 9-R-9-deazaguanine with cycloaliphatic substituents at the 9-position

Table 2. 2nd R's group and IC50

Number	Name	IC_{50}
41	3-Trifluoromethytcyclohexylmethyl	0.025 ± 0.006
42	3-Methylcyclohexytmethyl	0.025
43	Cyclopentylmethyl	0.029
44	Cycloheptylmethyl	0.03
45	Cyclohexylmethyl	0.047 ± 0.014
46	2-Tetrahydrofuranylmethyl	0.07
47	2-Adamantylmethyl	0.09
49	Cyclohexyl	1.3

3rd group: 9-(1-(3-chlorophenyl)-R)-9-deazaguanine (see in figure 3), R group see in table 3.

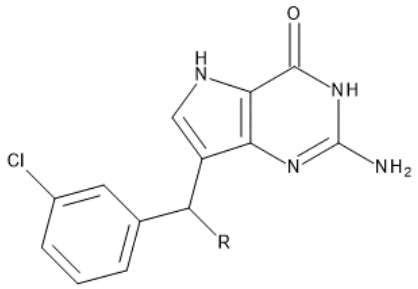

Figure 3. 9-(1-(3-chlorophenyl)-R)-9-deazaguanine

Table 3. 3rd R's group and IC_{50}.

Number	Name	IC_{50}
51	CH2CN	11 ± 2
53	CH2CO2Me	85

- Performed geometry optimization.
- Used ADT to merge non-polar hydrogen atoms, assign atomic charges, set torsion for flexible docking, and assign all necessary parameters for the docking calculations

3. Molecular docking calculation

   - Used Vega to calculate center of mass of inhibitor existed in the X-ray complex structure.

     Center mass of inhibitor in 6F4X is at coordinate (-12.11, 6.14, -15.96).

     Center mass of inhibitor in 6F4W is at coordinate (-12.78, 6.24, -16.16).

   - Used the center of mass of inhibitor as a center of grid box. The grid box size was set as 30 x 30 x 30 $Å^3$, because the search area should bigger than ligands size around 2 times of them size.

   - Got best binding affinity of docking calculates in each ligand (see in Table 4).

4. Result Analysis

   - Used Vega to extract the first rank docked structure, which has the best binding affinity.

   - Used DS Viewer to analyze enzyme-ligand interaction.

   - Compared and analyzed the binding modes between 6F4X and 6F4W.

Chapter 3

Results and discussions

3.1 Validation of the docking software

In molecular docking study, one frequently asked question is about the accuracy of the docking results, i.e., whether the docking software can predict the binding configuration close to the X-ray structure. Therefore, it is important to perform a validation of the docking software to assure the accuracy of the docking results. In this study, the X-ray complexed structure between HpPNP without phosphate and formycin A (its inhibitor), PDB code 6F4W, was used as testing model. Formycin A was extracted from the 6F4W structure and then it was docked back to the HpPNP. Then, the docked configuration was compared with the X-ray complexed structure. The docked configuration is quite similar to the X-ray one as shown in Figure 4. Therefore, accuracy of our docking calculation is acceptable.

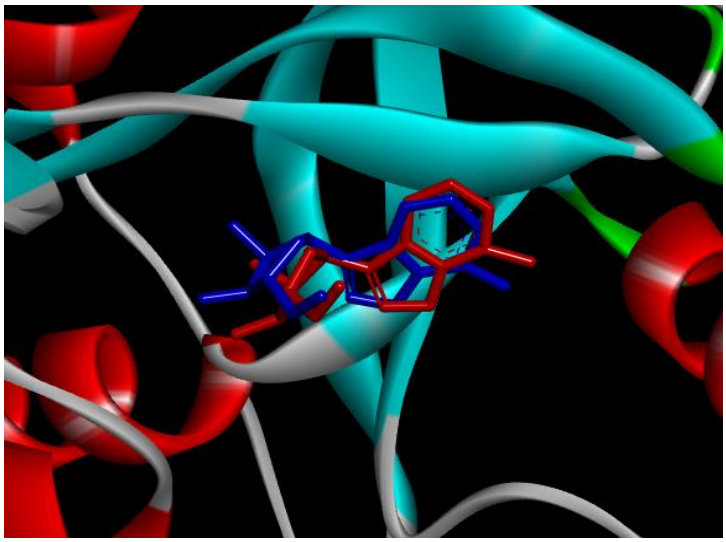

Figure 4. Comparison of formycin A configuration in the HpPNP between the X-ray structure (red) and the docked structure (blue).

3.2 Effect of phosphate on the binding configuration

In order to compare the difference of ligand binding configuration in HpPNP between model with (6F4X) and without (6F4W) phosphate, root means square deviation (RMSD) value was calculated for each ligand. RMSD value could told how close of docking position between docked ligands in both models, the lower the value the closer the docking position of ligands. Our results showed that RMSD values are scattered (see in Table 4). Most ligands bind differently to HpPNP with and without phosphate (high RMSD value), for example, ligand number 41 has the highest RMSD and it binds to HpPNP in a different position (see in Figure 4). On the other hand,

some ligands bind to HpPNP with and without phosphate quite similar (small RMSD value), for example, ligand number 53 has the lowest RMSD and it binds to HpPNP at almost the same position and orientation (see in Figure 5).

Table 4. Comparison data of RMSD between HpPNP model: 6F4X and 6F4W. IC_{50} of PO_4. Ratio shown IC_{50}(50 mM PO_4)/IC_{50} (1 mM PO_4). Affinity of binding (kcal/mol).

Ligand No.	IC_{50}	Model code		rmsd	ratio
		6f4x	6f4w		
21	20 ± 7	-8.6	-8.4	5.4717	8
23	23	-8.8	-8.4	0.2701	12
24	24	-8.7	-8.4	4.0078	9
27	25 ± 2.8	-8.1	-8.2	5.0156	8
28	36 ± 12	-9.5	-8.8	4.4277	5
30	57	-9.1	-8.7	1.4479	5
31	70 ± 20	-8.7	-8.3	6.6388	4
33	83	-9.2	-9.0	5.6011	4
37	260	-8.8	-8.4	5.1631	9
38	270	-8.9	-8.1	3.3737	7
41	0.025 ± 0.006	-8.2	-7.7	8.6691	32
42	0.025	-7.8	-7.8	6.8668	33
43	0.029	-7.9	-7.6	0.6923	62
44	0.03	-8.0	-7.9	5.4056	29
45	0.047 ± 0.014	-8.2	-8.4	2.8738	45
46	0.07	-7.5	-7.5	1.0772	4
47	0.09	-8.6	-8.8	7.2246	28
49	1.3	-8.0	-8.2	3.7018	110
51	11 ± 2	-8.1	-8.7	7.3827	136
53	85	-8.7	-8.7	0.2424	940

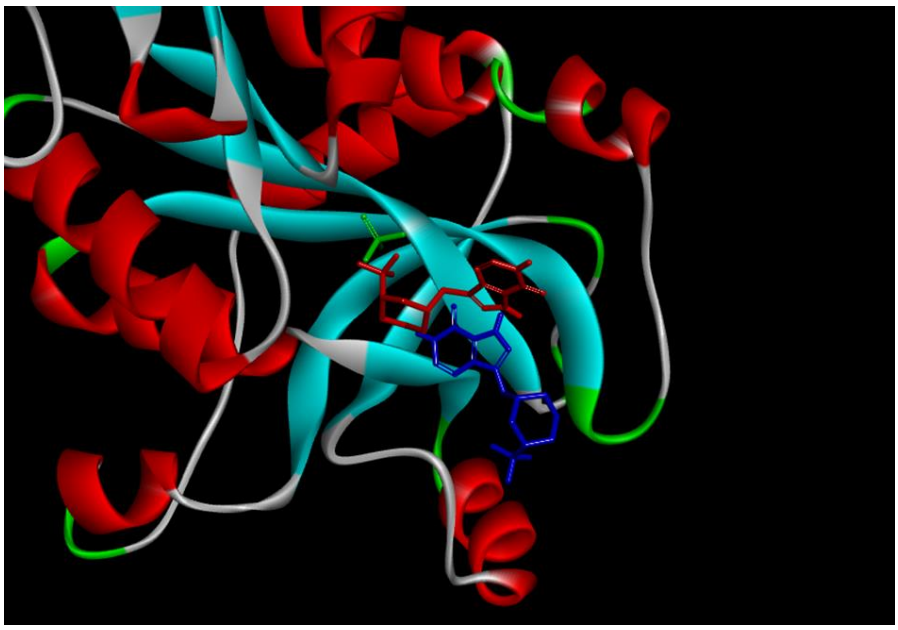

Figure 5. Composition of ligand No. 41. Blue molecule for model: 6F4X. Red molecule for model: 6F4W. Green molecule for PO4.

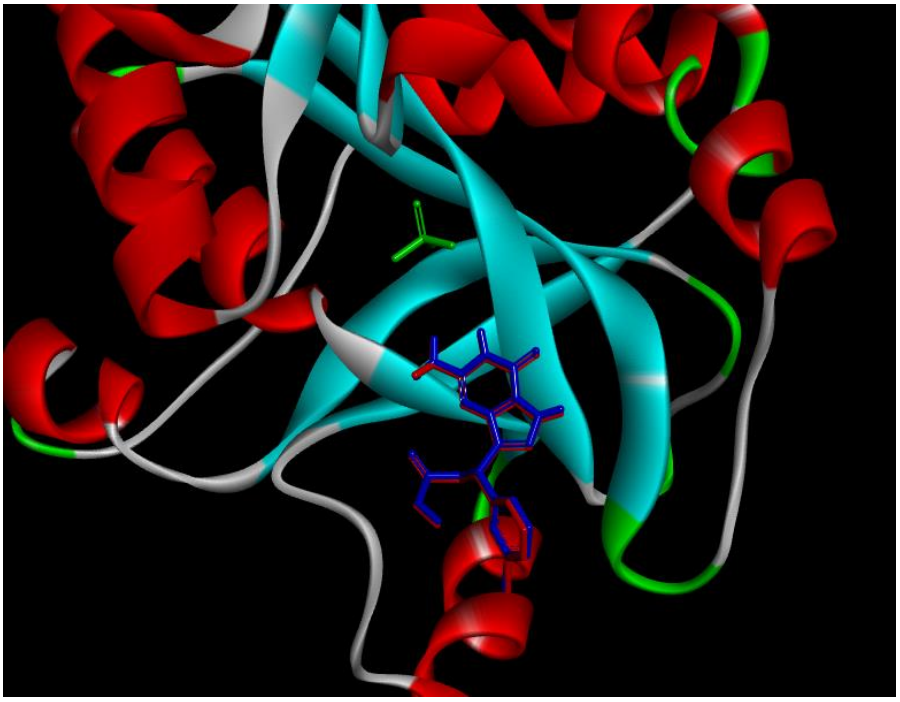

Figure 6. Composition of ligand number 53. Blue molecule for model: 6F4X. Red molecule for model: 6F4W. Green molecule for PO4.

There were 3 amino acids in HpPNP both with and without phosphate that have interactions with ligands. PHE159 has interaction with every ligand (see in Table 5). THR90 and LEU206 interacts with most ligands (see in Table 5).

Table 5. Interaction of ligand's 1st group and amino acid in HpPNP. Highlight in orange color for interaction with model: 6F4X. Highlight in pink color for interaction with model: 6F4W.

compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D:43
21	20 ± 7	6f4x	✓	x	x	✓	✓	x	x	✓	✓	✓	✓
21	x	6f4w	✓	x	x	✓	✓	✓	x	x	x	x	✓
23	23		✓	✓	✓	✓	x	✓	✓	x	✓	x	x
23	x		✓	✓	✓	✓	x	✓	✓	x	✓	x	x
24	24		✓	x	x	✓	x	x	x	✓	✓	✓	✓
24	x		✓	✓	✓	✓	x	✓	✓	✓	✓	x	x
27	25 ± 2.8		x	✓	✓	✓	✓	✓	x	x	✓	✓	✓
27	x		✓	x	x	✓	✓	x	✓	✓	x	x	✓
28	36 ± 12		✓	x	x	✓	✓	x	x	x	✓	✓	✓
28	x		✓	✓	✓	✓	x	✓	✓	✓	✓	x	x
30	57		✓	✓	✓	✓	x	✓	✓	x	✓	x	x
30	x		✓	✓	✓	✓	x	✓	✓	x	✓	x	x
31	70 ± 20		✓	x	x	✓	✓	x	✓	✓	x	✓	✓
31	x		x	✓	✓	✓	✓	✓	x	x	x	x	✓
33	83		✓	x	x	✓	x	x	x	✓	✓	✓	✓
33	x		✓	x	x	✓	x	✓	x	✓	x	x	✓
37	260		✓	x	x	✓	✓	✓	x	x	x	✓	✓
37	x		✓	x	✓	✓	✓	✓	x	x	x	x	✓
38	270		✓	x	✓	✓	✓	x	x	✓	✓	✓	✓
38	x		✓	x	x	✓	x	✓	✓	x	✓	x	✓

From the data in Table 5, it is not certain to tell that phosphate (PO_4) had effect on docking efficiencies or not. We know that phosphate is one of the substrates in model code 6F4X, to be compare with model 6F4W the docking position will clearly difference. As shown in figure 4 the position of ligand in 6F4X and 6F4W shown that the one with phosphate the composition of ligand will turn away from phosphate, while model without phosphate ligand will dock in position of phosphate (see in Figure 4).

As the first R's group of ligands, most interacted with phosphate in model 6F4X, but docked ligand in model 6F4W had no interact with phosphate (see in Table 5).

The second R's group of ligands and third R's group of ligands had no interaction with phosphate in both models as shown in Table 6, and Table 7.

Table 6. Interaction of ligand's 2nd group and amino acid in HpPNP. Highlight in orange color for interaction with model: 6F4X. Highlight in pink color for interaction with model: 6F4W.

compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D: 43
41	0.025 ± 0.006	6f4x	✓	✓	✓	✓	x	x	x	x	✓	x	x
41	x	6f4w	✓	x	x	✓	✓	✓	x	x	✓	x	✓
42	0.025		✓	✓	✓	✓	x	x	✓	x	✓	x	x
42	x		✓	x	x	✓	✓	✓	x	x	✓	x	✓
43	0.029		✓	✓	✓	✓	x	✓	x	x	✓	x	x
43	x		✓	x	x	✓	✓	✓	x	x	✓	x	x
44	0.03		✓	✓	✓	✓	x	x	x	x	✓	x	x
44	x		✓	✓	x	✓	x	✓	x	x	✓	x	✓
45	0.047 ± 0.014		✓	✓	✓	✓	x	✓	x	x	✓	x	x
45	x		✓	✓	x	✓	✓	x	✓	✓	✓	x	x
46	0.07		✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x
46	x		✓	x	x	✓	x	✓	✓	x	x	x	✓
47	0.09		✓	✓	✓	✓	x	x	x	x	✓	x	x
47	x		x	x	x	✓	✓	✓	x	x	x	x	x
49	1.3		✓	✓	✓	✓	✓	x	x	✓	✓	x	x
49	x		✓	x	x	✓	x	x	x	x	x	x	✓

Table 7. Interaction of ligand's 3rd group and amino acid in HpPNP. Highlight in orange color for interaction with model: 6F4X. Highlight in pink color for interaction with model: 6F4W.

compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D: 43
51	11 ± 2	6f4x	✓	x	x	✓	x	x	x	x	✓	x	✓
51	x	6f4w	x	x	x	✓	✓	✓	✓	x	✓	x	✓
53	85		✓	✓	✓	✓	x	x	✓	x	✓	x	x
53	x		x	x	x	✓	✓	✓	✓	x	✓	x	✓

The result of phosphate indicates that it cannot confirm that phosphate had effect with docking efficiency, it just one of the substrates in 6F4X. The binding site of phosphate to active site of PNP in H. Pylori does not cause a conformational change by itself, thus it just added more piece of puzzle in the sophisticated catalyzed mechanism and complex cooperation between subunits of PNP only [2].

But this did not mean phosphate did not have an effect at all. From the resulted the researchers got; it tells that most of ligands with same model are not docking in the same place. The reason is even 6F4X and 6F4W have the same model but there is one different that 6F4X has phosphate but 6F4W not, so phosphate might have some effect to the place of docking as well (see in figure 4). The most of docked ligand in model 6F4X had an effect with phosphate that phosphate will pull ligand away from its position as ligand number 41 (see in figure 4). The docked

ligands in model 6F4W will had no effect with phosphate as ligand number 41 (see in figure 4) and it will be docked near or overlap phosphate molecule.

The interaction shows us that all of ligand have PHE A: 159, most of ligands have THR A: 90 and LEU A:206, for example figure 6, and figure 7. These three amino acids represent the most compared to other amino acid. The papers show us that after docking, they also have these three amino acids too so that could assume that our docking result are right [3].

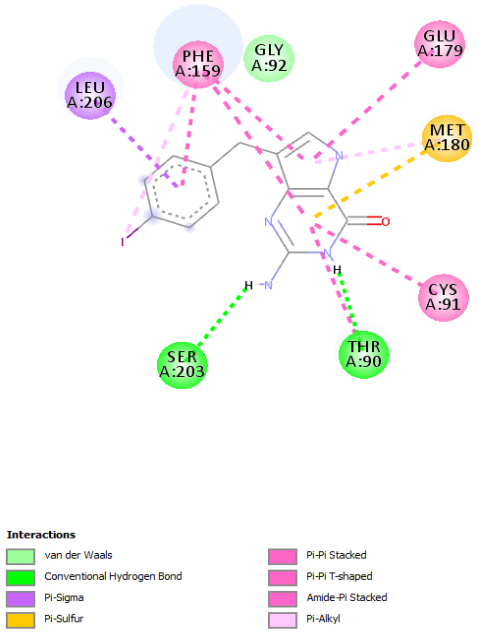

Figure 7. Interaction of ligand number 23 and amino acid in HpPNP model: 6F4X.

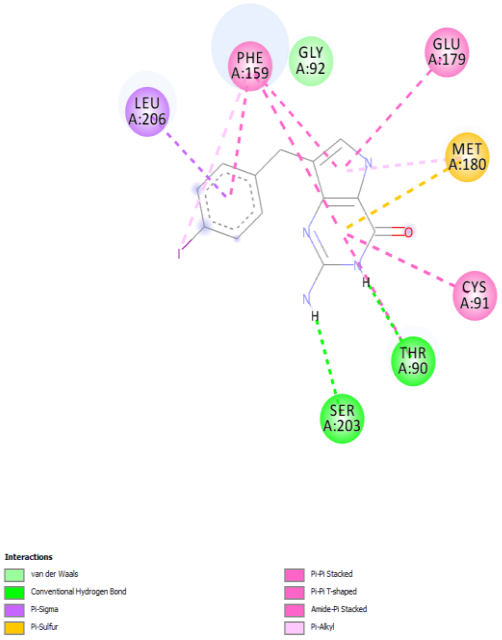

Figure 8. Interaction of ligand number 23 and amino acid in HpPNP model: 6F4W.

Another point is ligand number 41-53 (see in appendix) has no interaction with phosphate in both models. The reason is program had computed that to be more docking efficient in the active site of the enzyme, ligands will be docked in position that not interacted with phosphate.

Chapter 4

Conclusion

In summary, molecular docking calculations of 20 inhibitors to PNP enzyme with and without phosphate in the active site were performed to investigate the role of phosphate binding in the mechanism of this enzyme. The results showed that the phosphate binding does not have significant influence on important interactions between inhibitor and enzyme. However, the phosphate binding caused a shift in docked position of some inhibitors. With phosphate binding, some inhibitors bound to the active site a bit away from the phosphate position whereas these inhibitors occupied the space of phosphate binding position when there is no phosphate.

Reference

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Appendices

Appendix A: List of interaction

A.1 Interaction in 1st group of ligands.

compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D:43
21	20 ± 7	6f4x	√	x	x	√	√	x	x	√	√	√	√
21	x	6f4w	√	x	x	√	√	√	x	x	x	x	√
23	23		√	√	√	√	x	√	√	x	√	x	x
23	x		√	√	√	√	x	√	√	x	√	x	x
24	24		√	x	x	√	x	x	x	√	√	√	√
24	x		√	√	√	√	x	√	√	√	√	x	x
27	25 ± 2.8		x	√	√	√	√	√	x	x	√	√	√
27	x		√	x	x	√	√	x	√	√	x	x	√
28	36 ± 12		√	x	x	√	√	x	x	x	√	√	√
28	x		√	√	√	√	x	√	√	√	√	x	x
30	57		√	√	√	√	x	√	√	x	√	x	x
30	x		√	√	√	√	x	√	√	x	√	x	x
31	70 ± 20		√	x	x	√	√	x	√	√	x	√	√
31	x		x	√	√	√	√	√	x	x	x	x	√
33	83		√	x	x	√	x	x	x	√	√	√	√
33	x		√	x	x	√	x	√	x	√	x	x	√
37	260		√	x	x	√	√	√	x	x	x	√	√
37	x		√	x	√	√	√	√	x	x	x	x	√
38	270		√	x	√	√	√	x	x	√	√	√	√
38	x		√	x	x	√	x	√	√	x	√	x	√

A.2 Interaction in 2nd group of ligands.

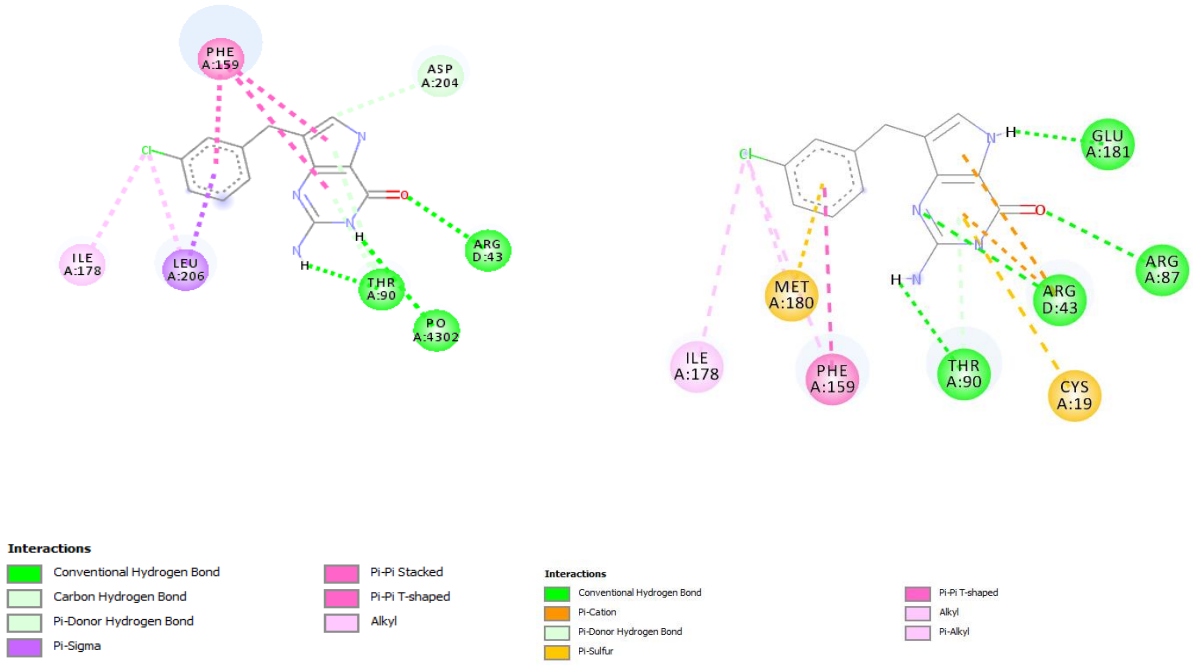
compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D:43
41	0.025 ± 0.006	6f4x	√	√	√	√	x	x	x	x	√	x	x
41	x	6f4w	√	x	x	√	√	√	x	x	√	x	√
42	0.025		√	√	√	√	x	x	√	x	√	x	x
42	x		√	x	x	√	√	√	x	x	√	x	√
43	0.029		√	√	√	√	x	√	x	x	√	x	x
43	x		√	x	x	√	√	√	x	x	√	x	x
44	0.03		√	√	√	√	x	x	x	x	√	x	x
44	x		√	√	x	√	x	√	x	x	√	x	√
45	0.047 ± 0.014		√	√	√	√	x	√	x	x	√	x	x
45	x		√	√	x	√	√	x	√	√	√	x	x
46	0.07		√	√	√	√	√	√	√	√	√	x	x
46	x		√	x	x	√	x	√	√	x	x	x	√
47	0.09		√	√	√	√	x	x	x	x	√	x	x
47	x		x	x	x	√	√	√	x	x	x	x	x
49	1.3		√	√	√	√	√	x	x	√	√	x	x
49	x		√	x	x	√	x	x	x	x	x	x	√

A.3 Interaction in 3rd group of ligands.

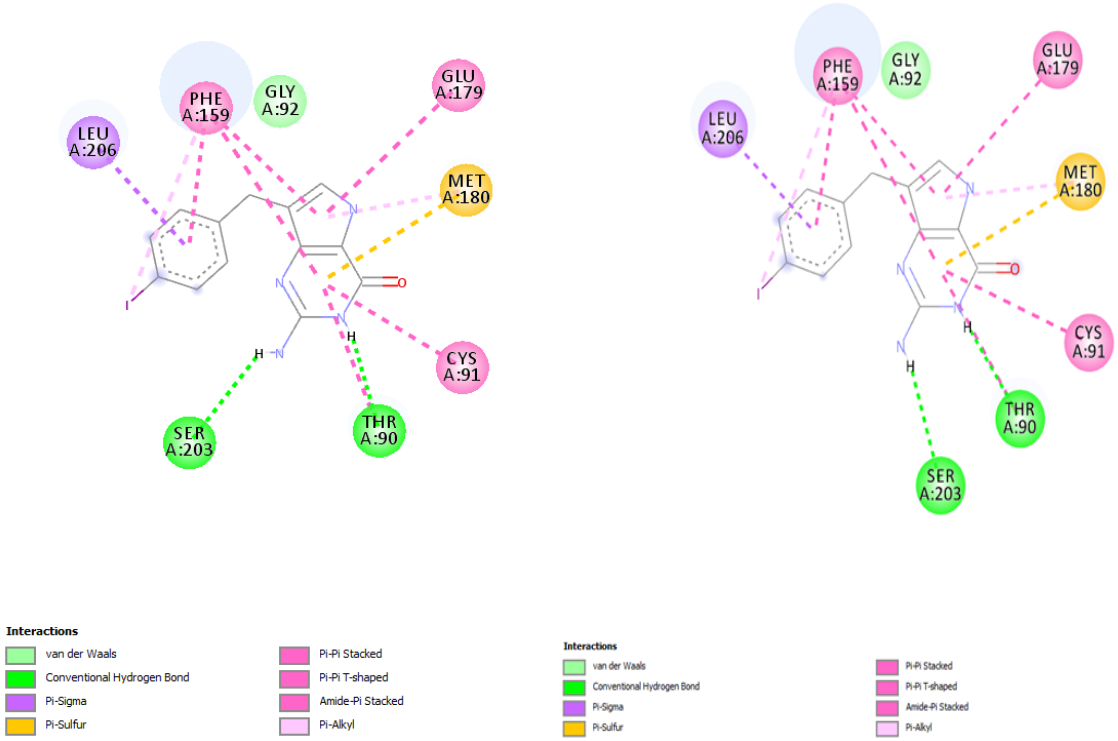
compound	IC50		THR A: 90	CYS A: 91	GLY A: 92	PHE A: 159	ILE A: 178	MET A: 180	SER A: 203	ASP A: 204	LEU A: 206	PO A: 4302	ARG D:43
51	11 ± 2	6f4x	√	x	x	√	x	x	x	x	√	x	√
51	x	6f4w	x	x	x	√	√	√	√	x	√	x	√
53	85		√	√	√	√	x	x	√	x	√	x	x
53	x		x	x	x	√	√	√	√	x	√	x	√

Appendix B: Interaction between HpPNP and its inhibitors

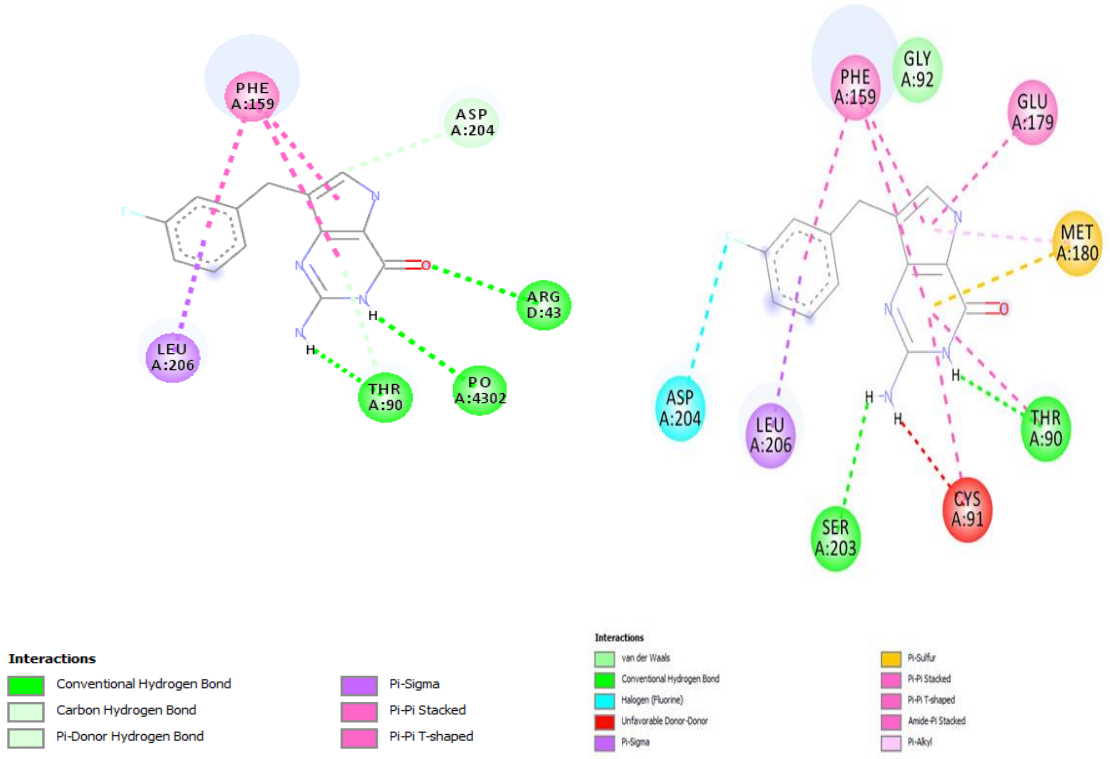
Ligand number 21, left 6F4x and right 6F4W



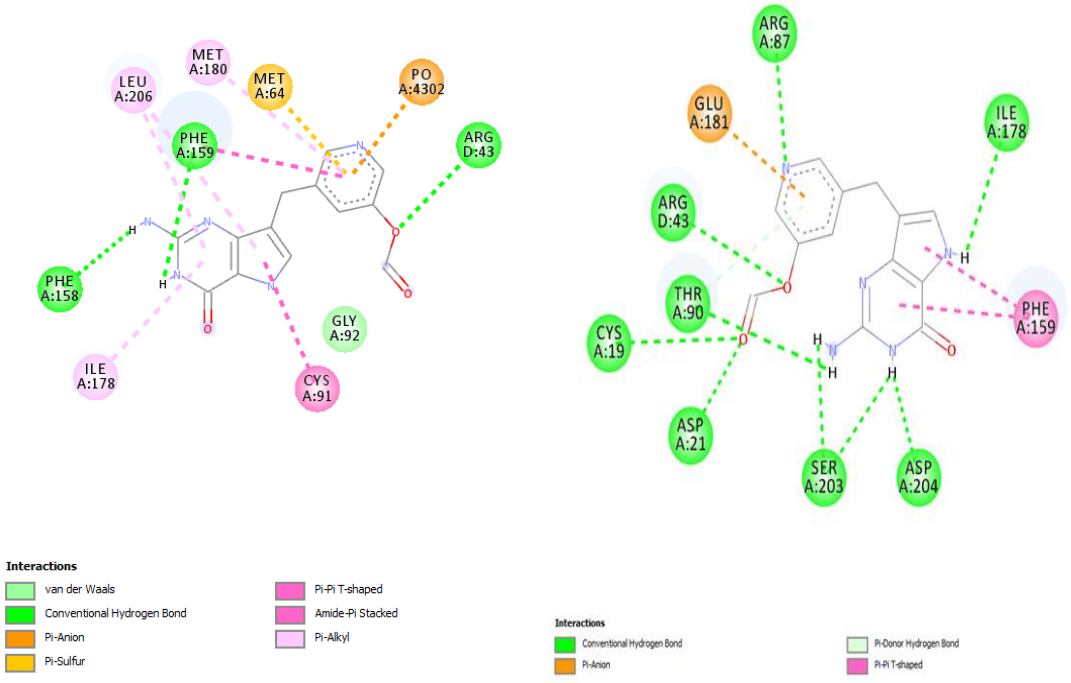
Ligand number 23, left 6F4x and right 6F4W



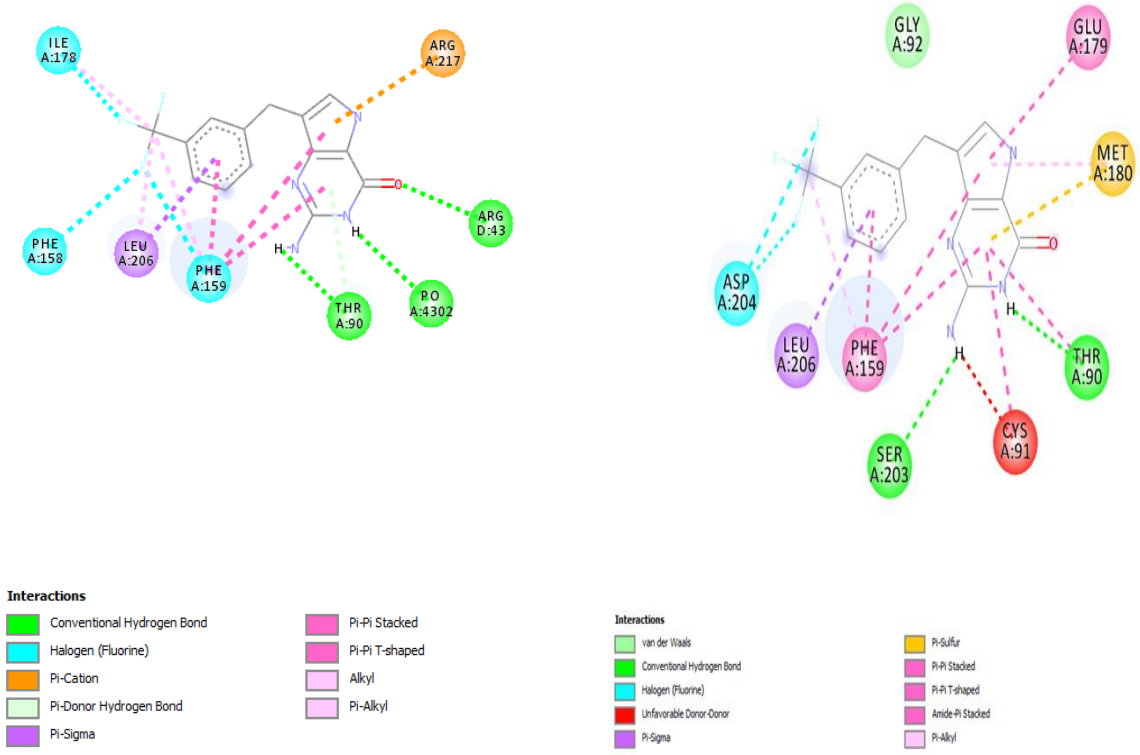
Ligand number 24, left 6F4x and right 6F4W



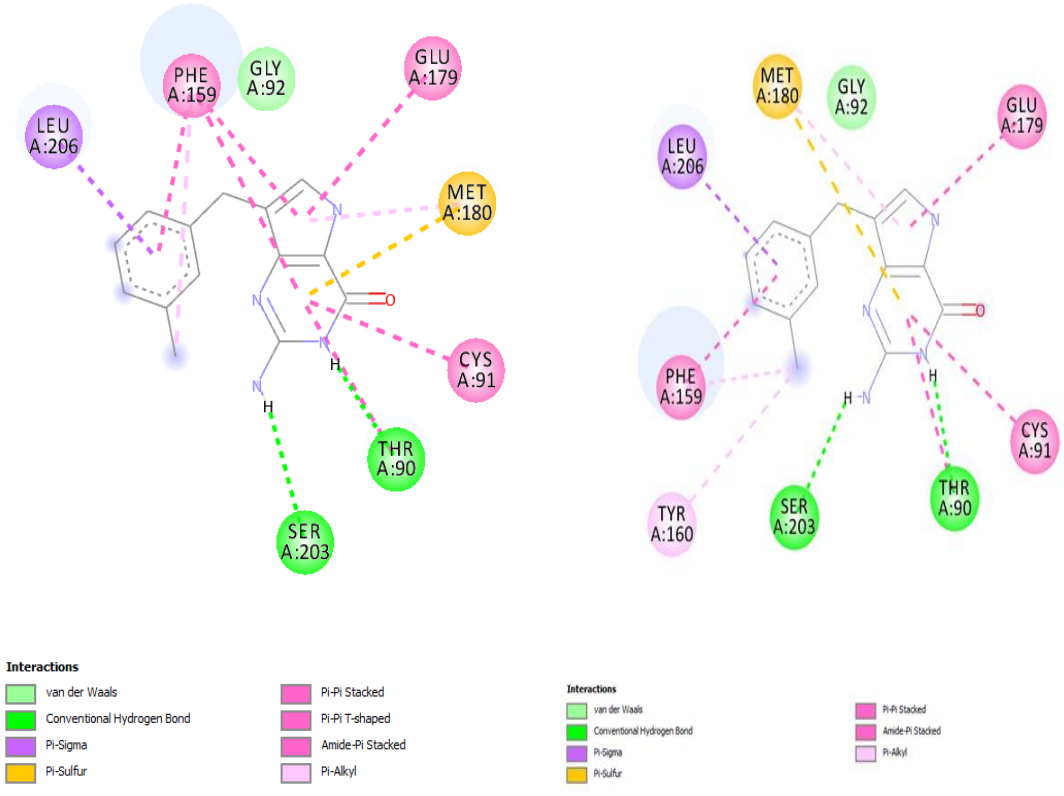
Ligand number 27, left 6F4x and right 6F4W



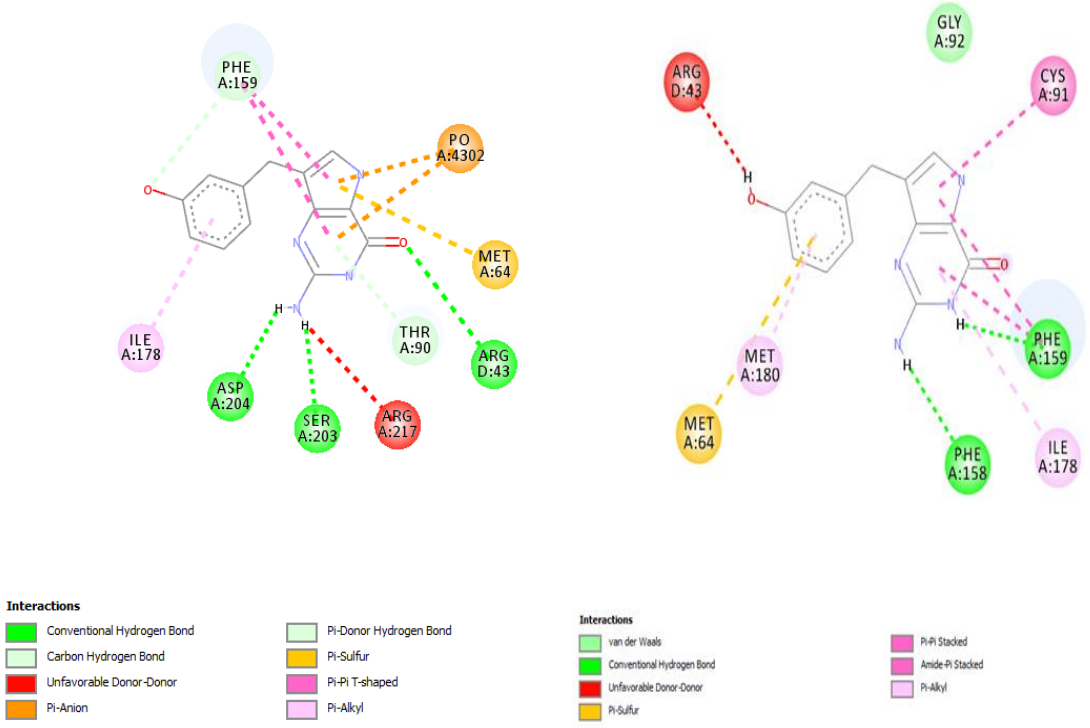
Ligand Number 28, left 6F4x and right 6F4W



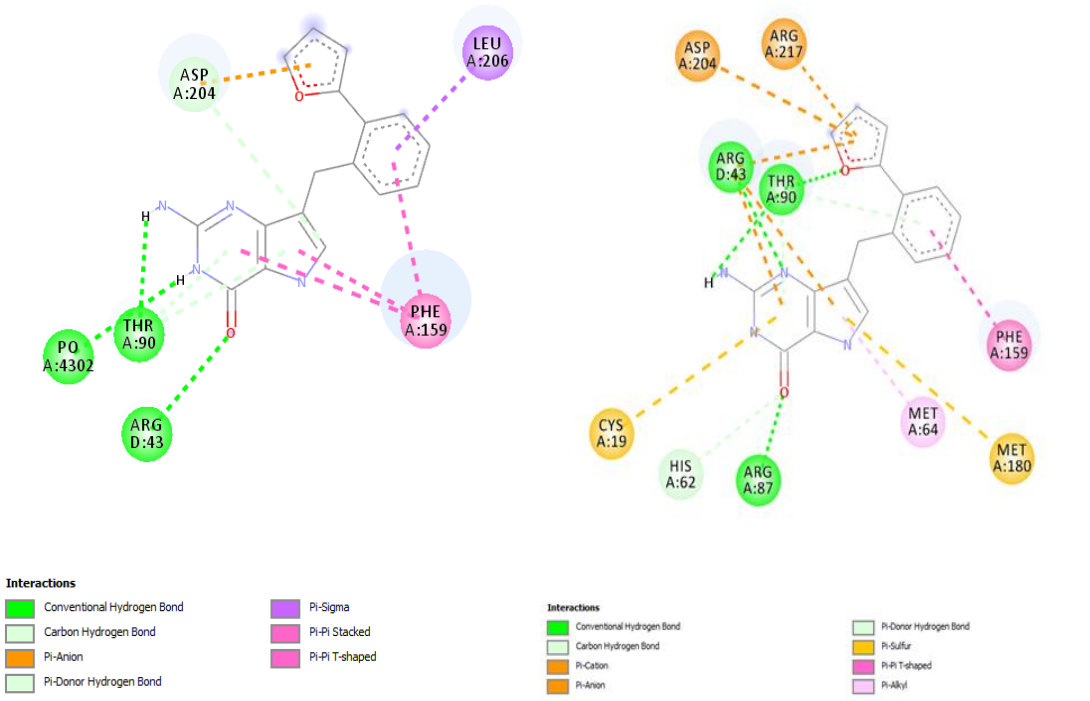
Ligand number 30, left 6F4x and right 6F4W



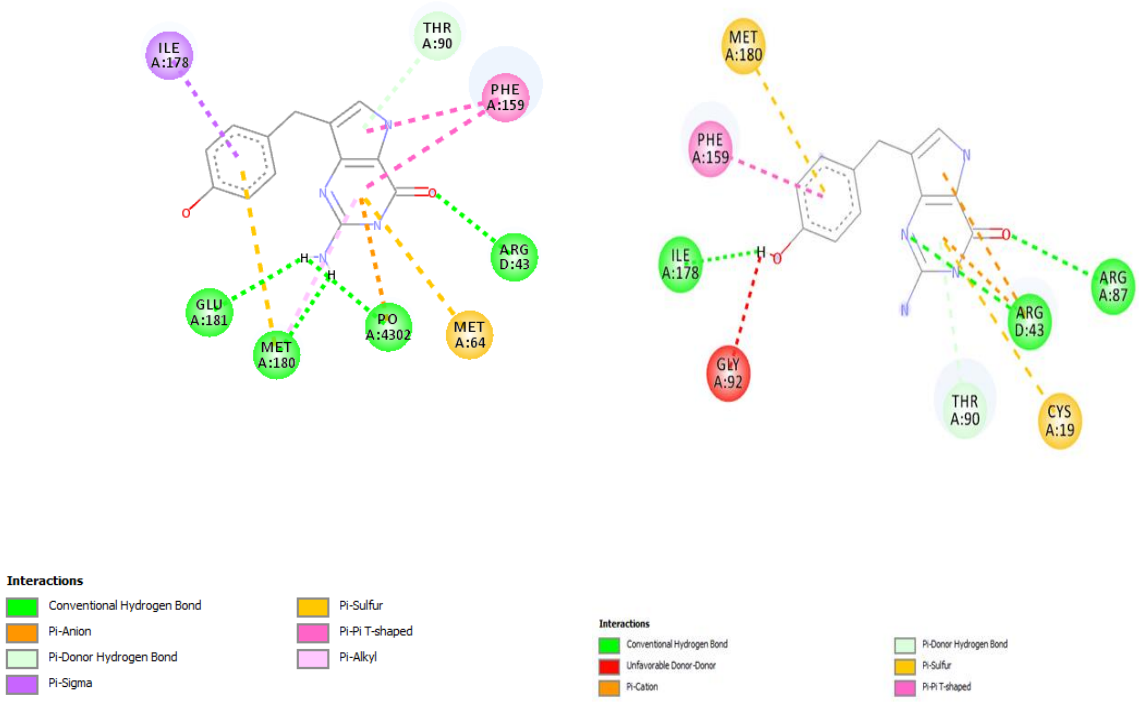
Ligand number 31, left 6F4x and right 6F4W



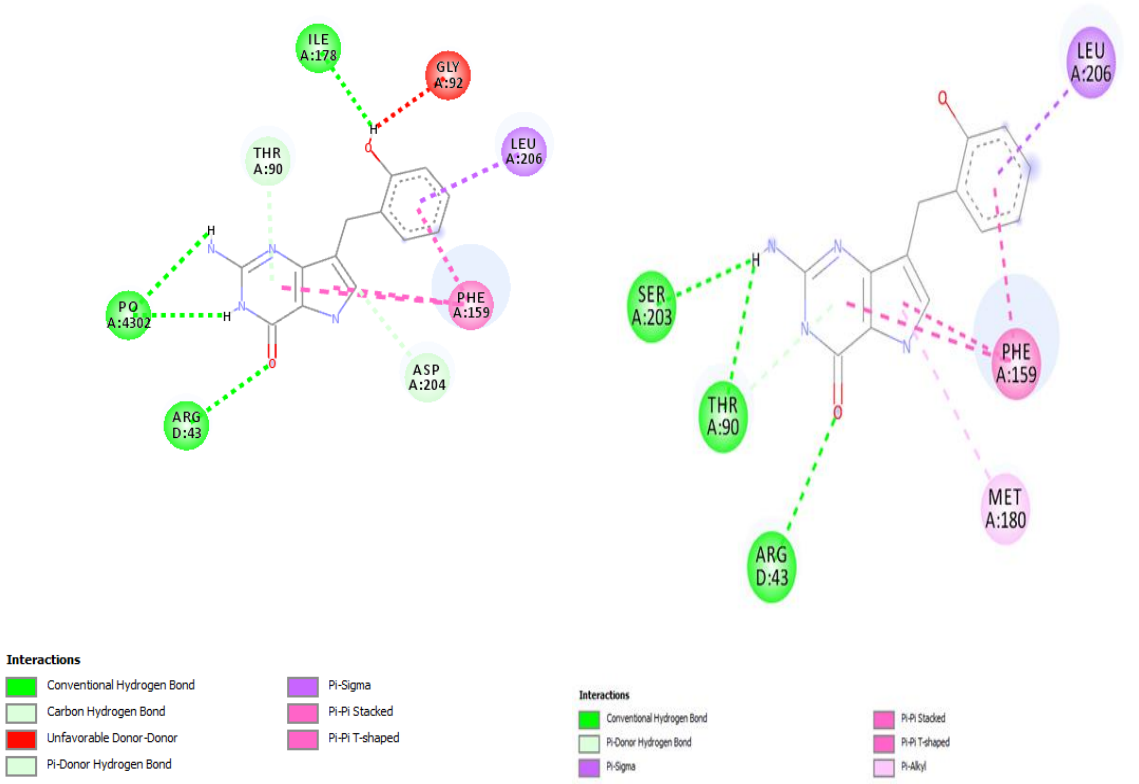
Ligand number 33, left 6F4x and right 6F4W



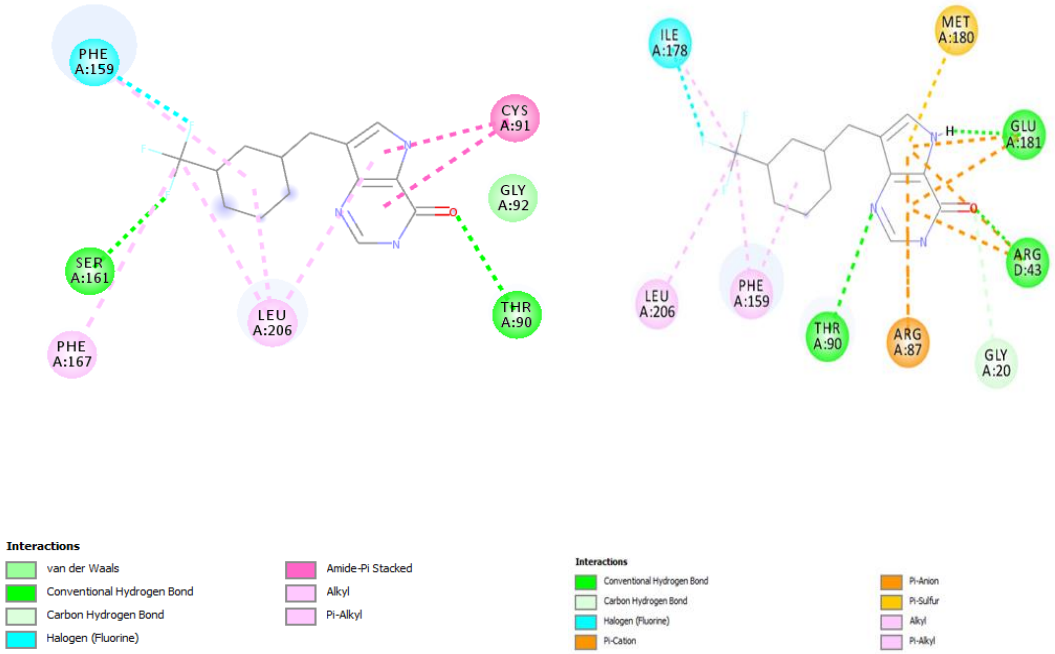
Ligand number 37, left 6F4x and right 6F4W



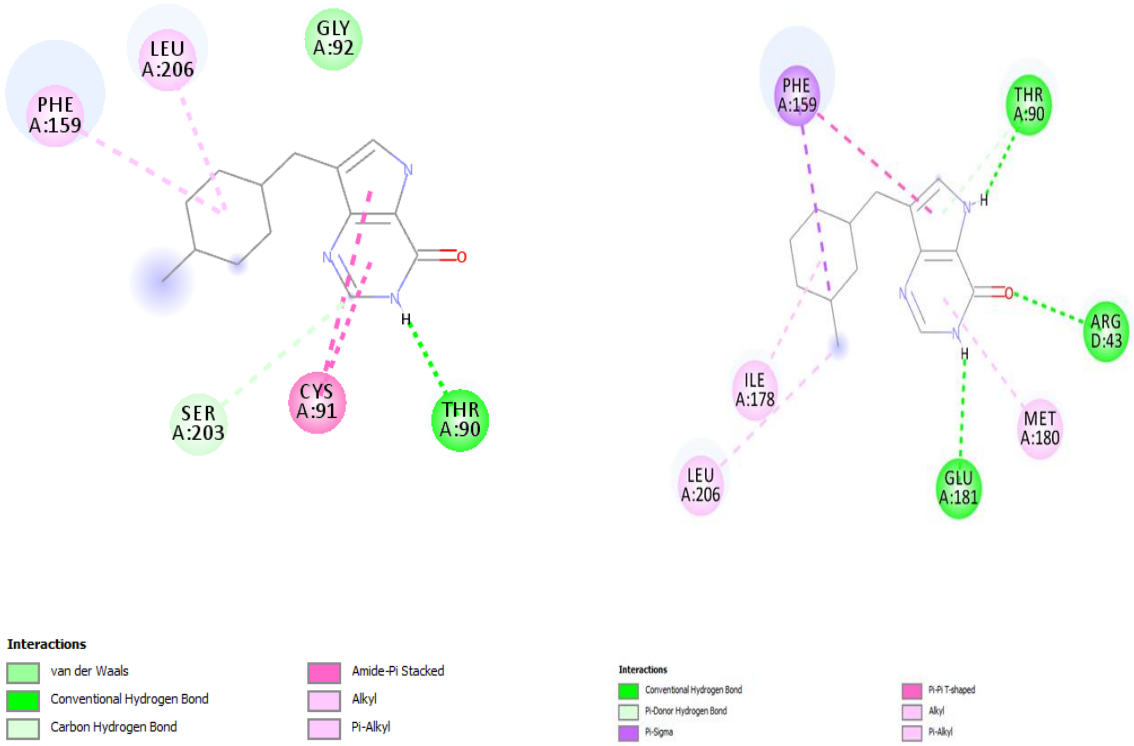
Ligand number 38, left 6F4x and right 6F4W



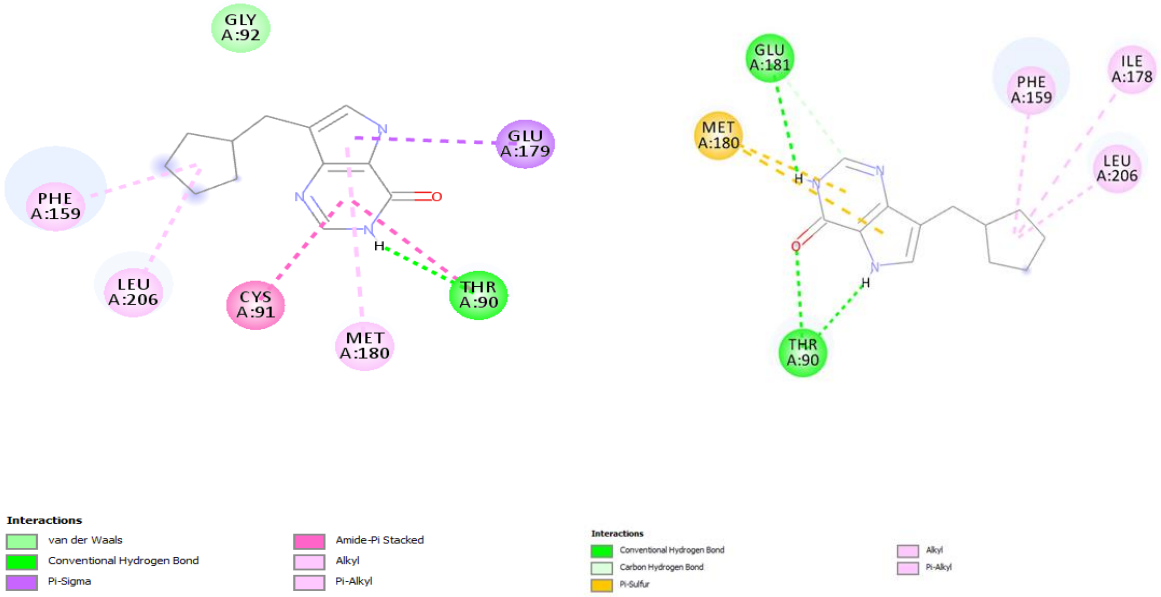
Ligand number 41, left 6F4x and right 6F4W



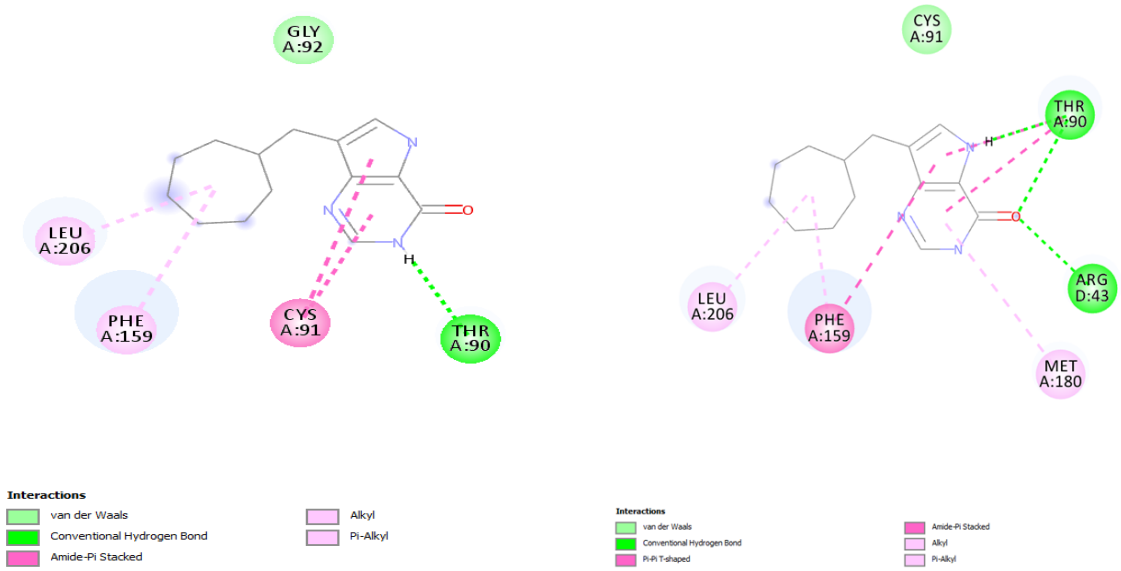
Ligand number 42, left 6F4x and right 6F4W



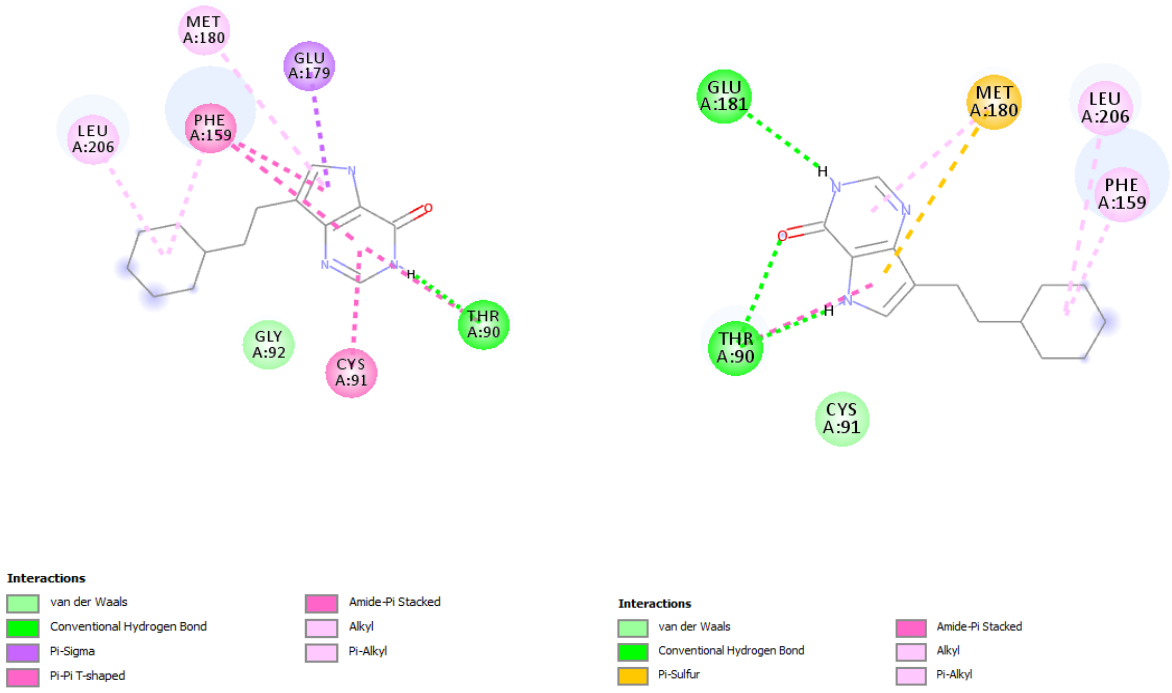
Ligand number 43, left 6F4x and right 6F4W



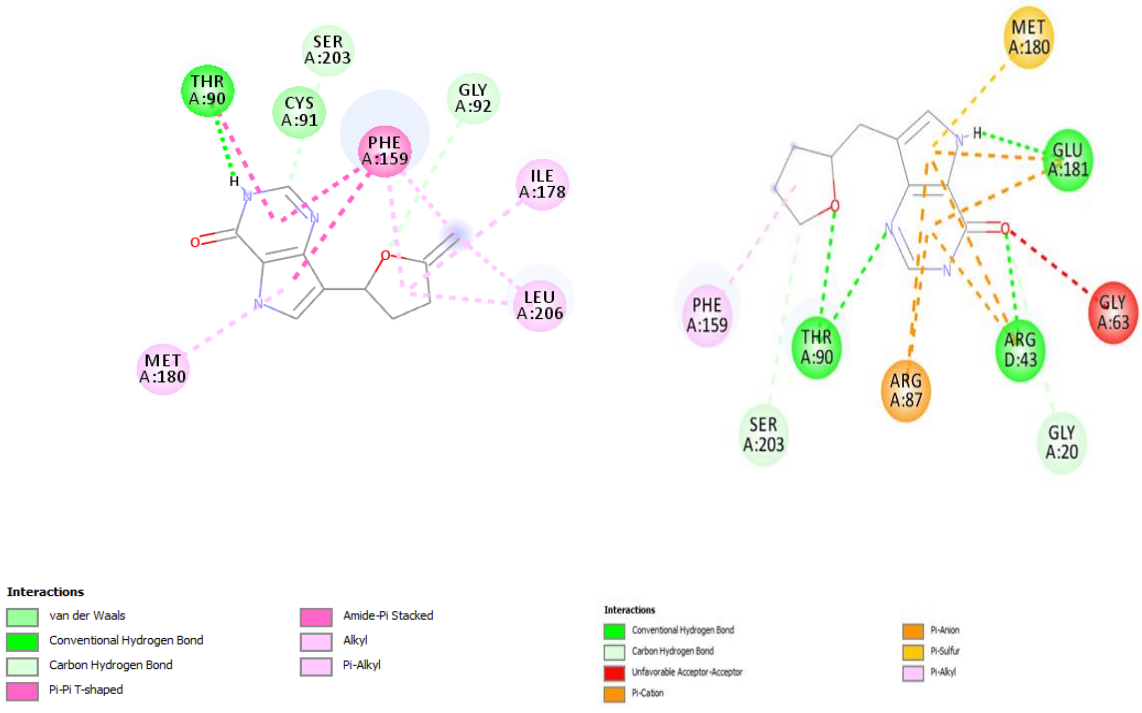
Ligand number 44, left 6F4x and right 6F4W



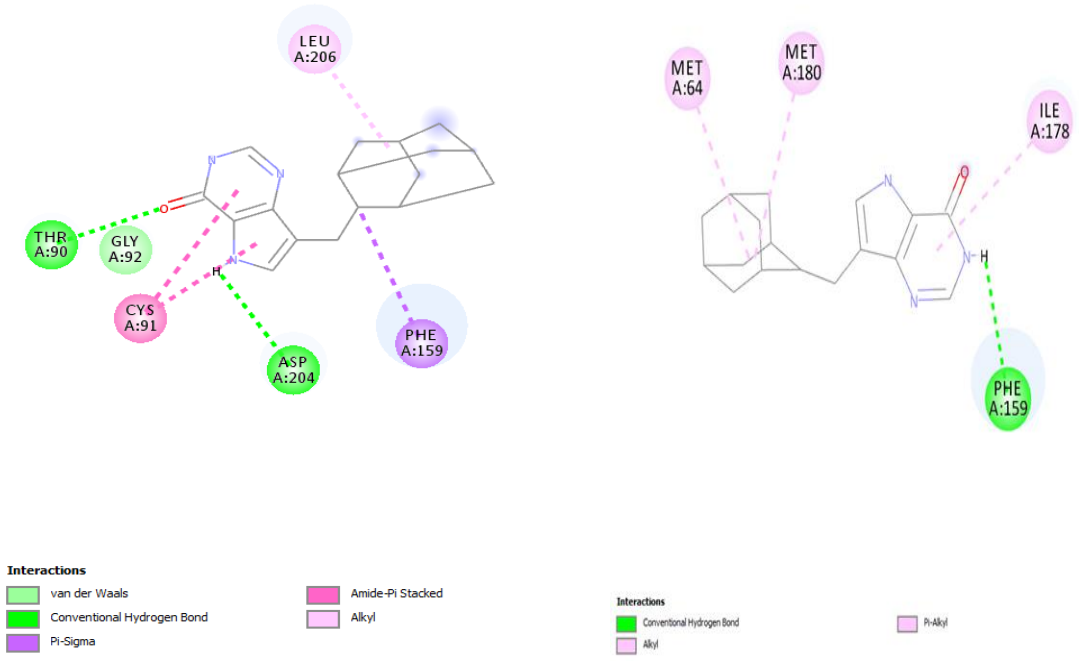
Ligand number 45, left 6F4x and right 6F4W



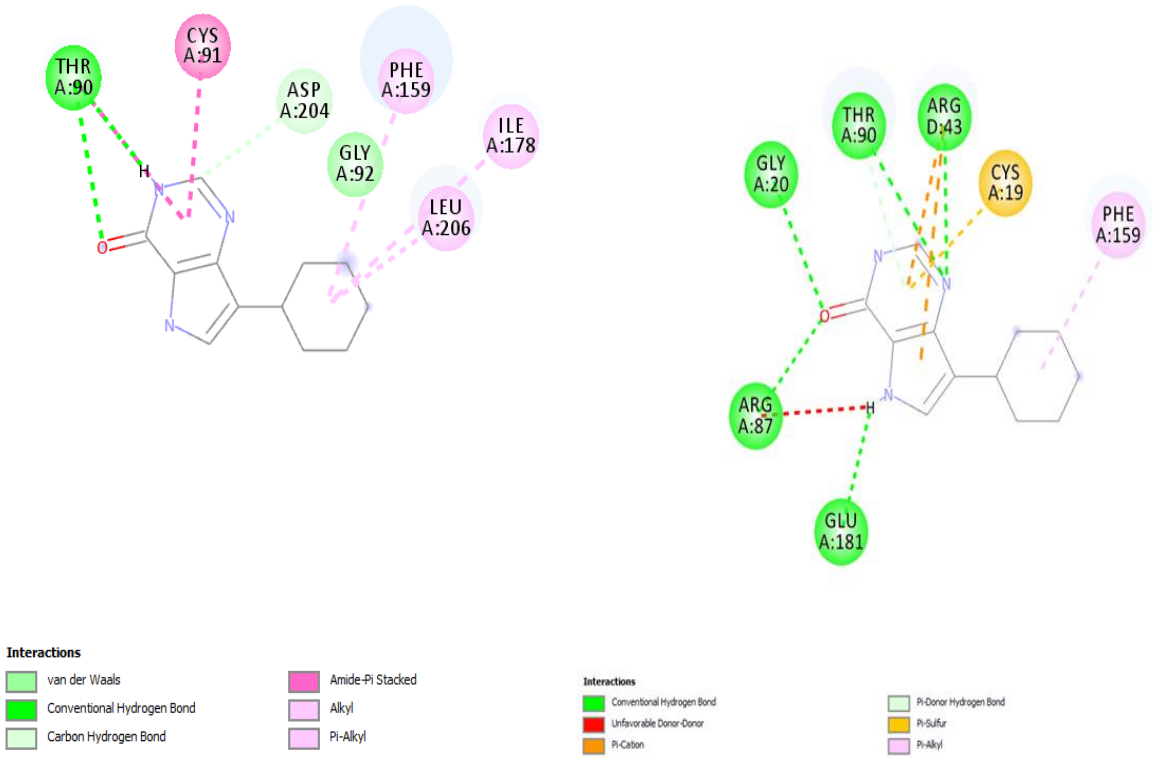
Ligand number 46, left 6F4x and right 6F4W



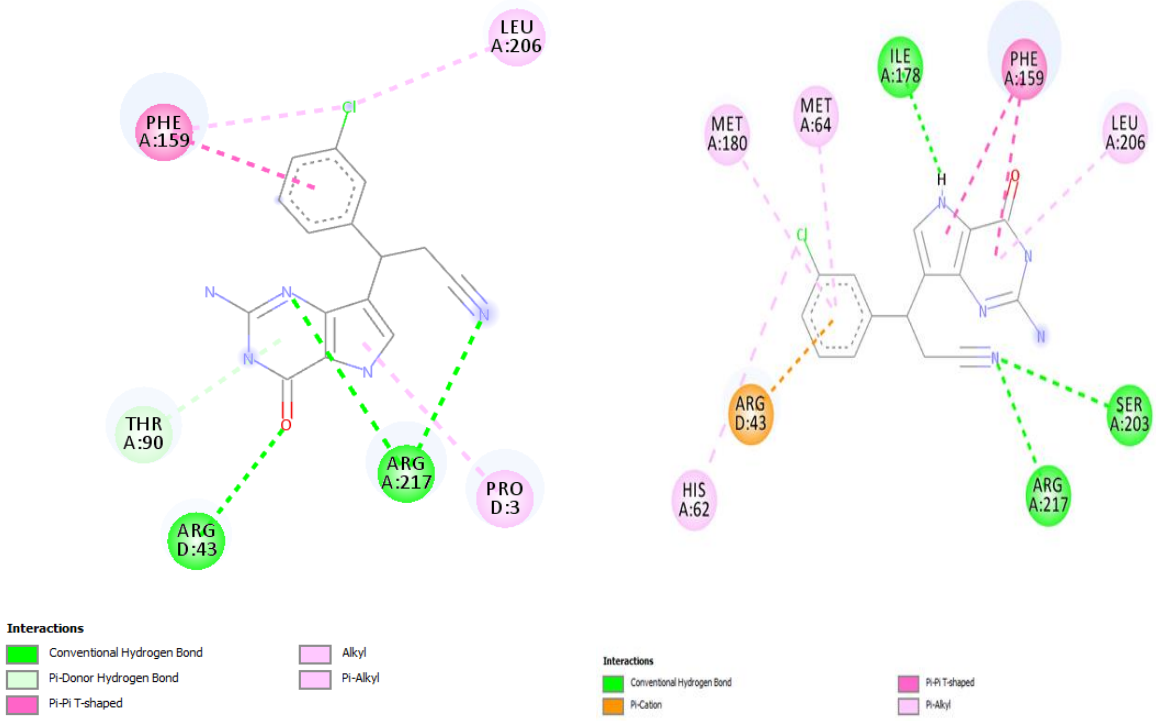
Ligand number 47, left 6F4x and right 6F4W



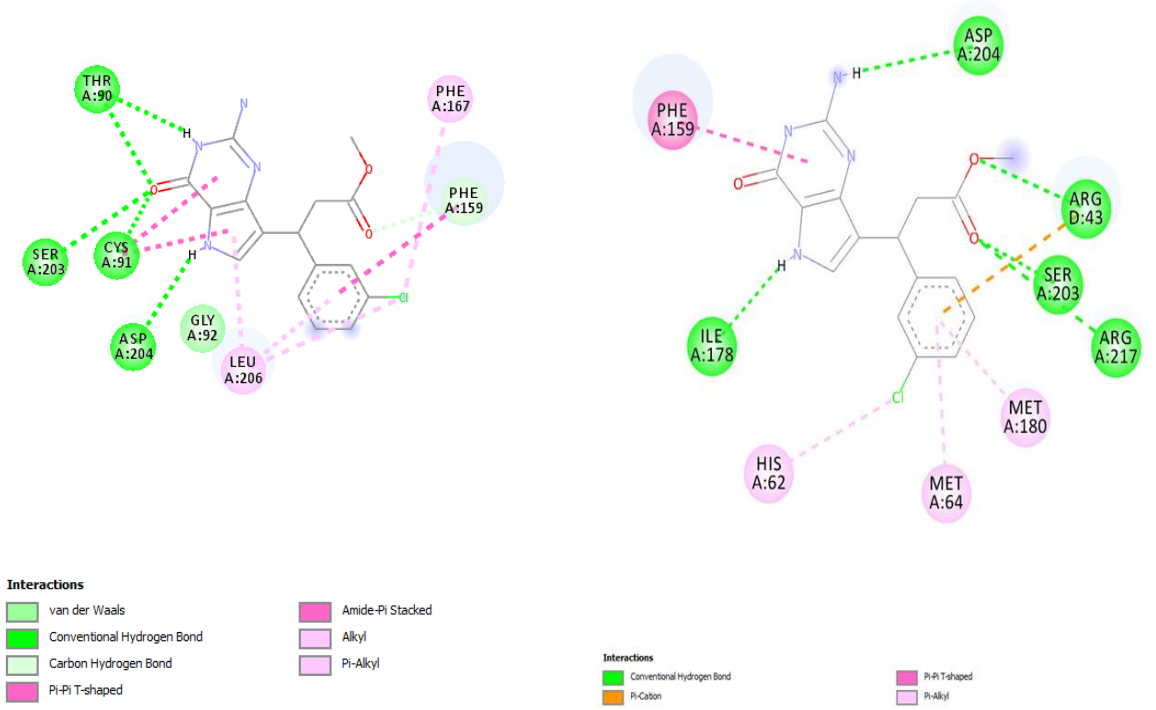
Ligand number 49, left 6F4x and right 6F4W



Ligand number 51, left 6F4x and right 6F4W

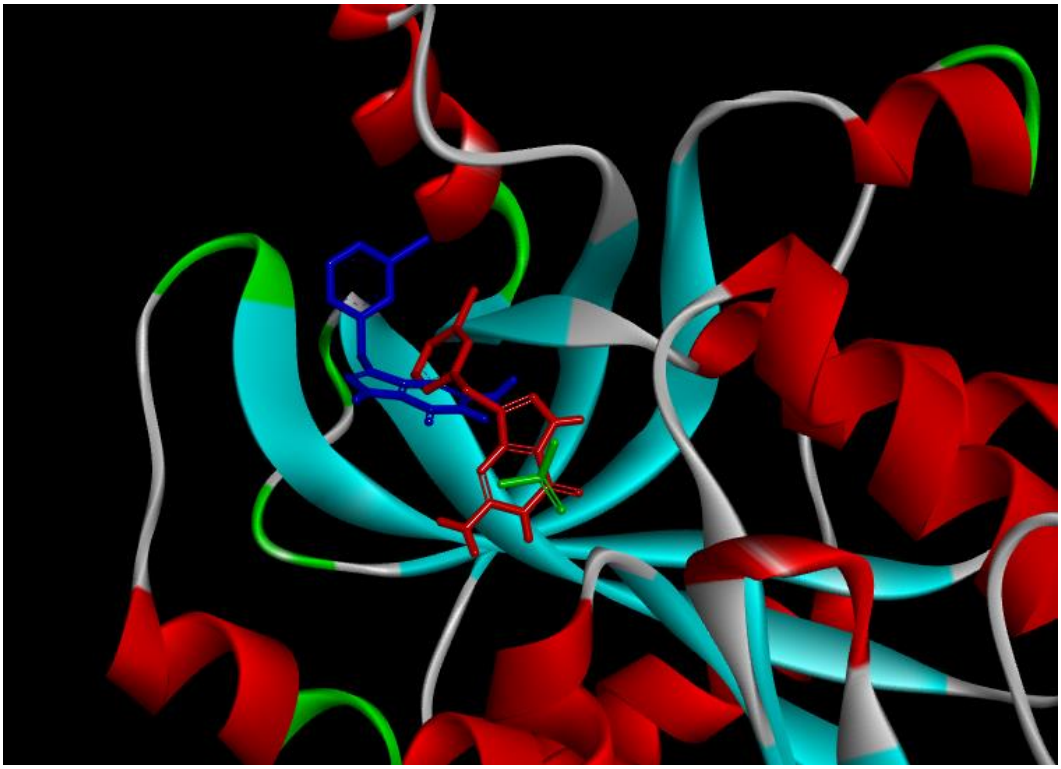


Ligand number 53, left 6F4x and right 6F4W

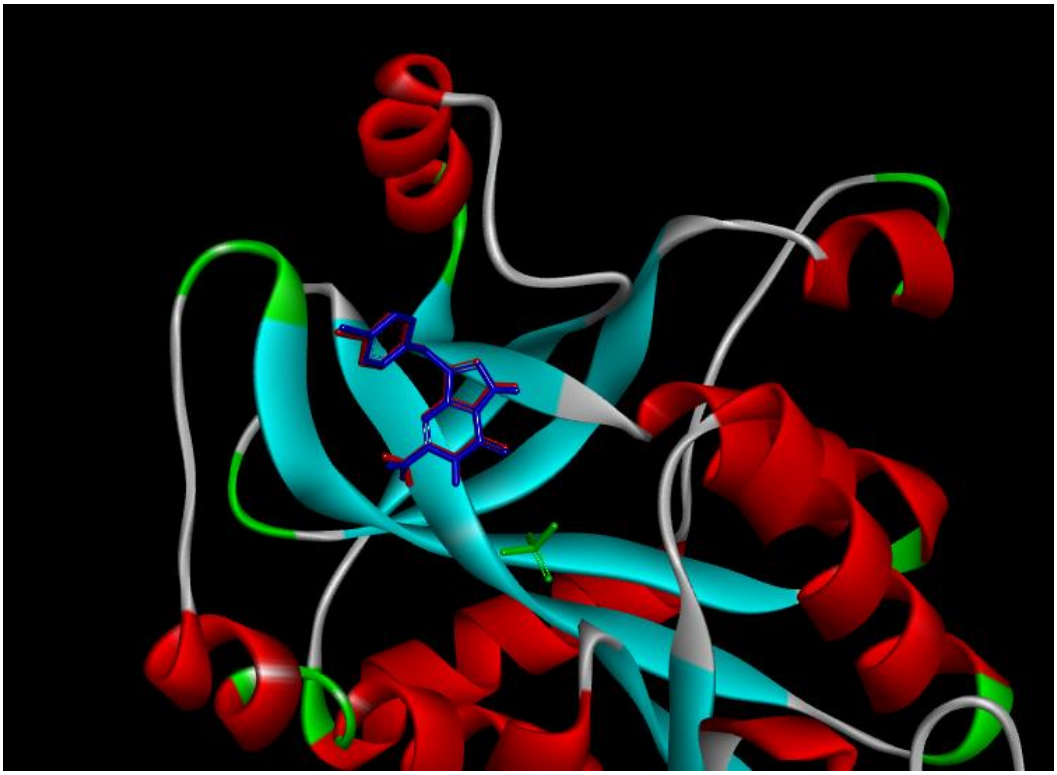


Appendix C: Comparison of interaction between model: 6F4X and 6F4W.

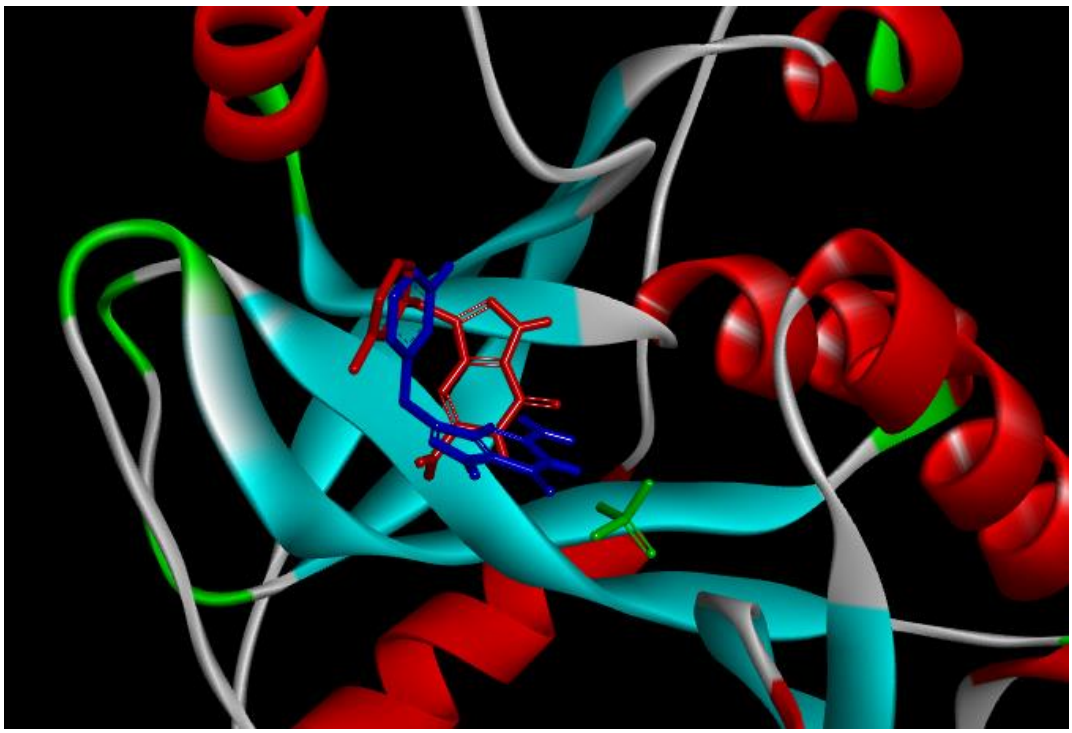
Comparison of ligand number 21



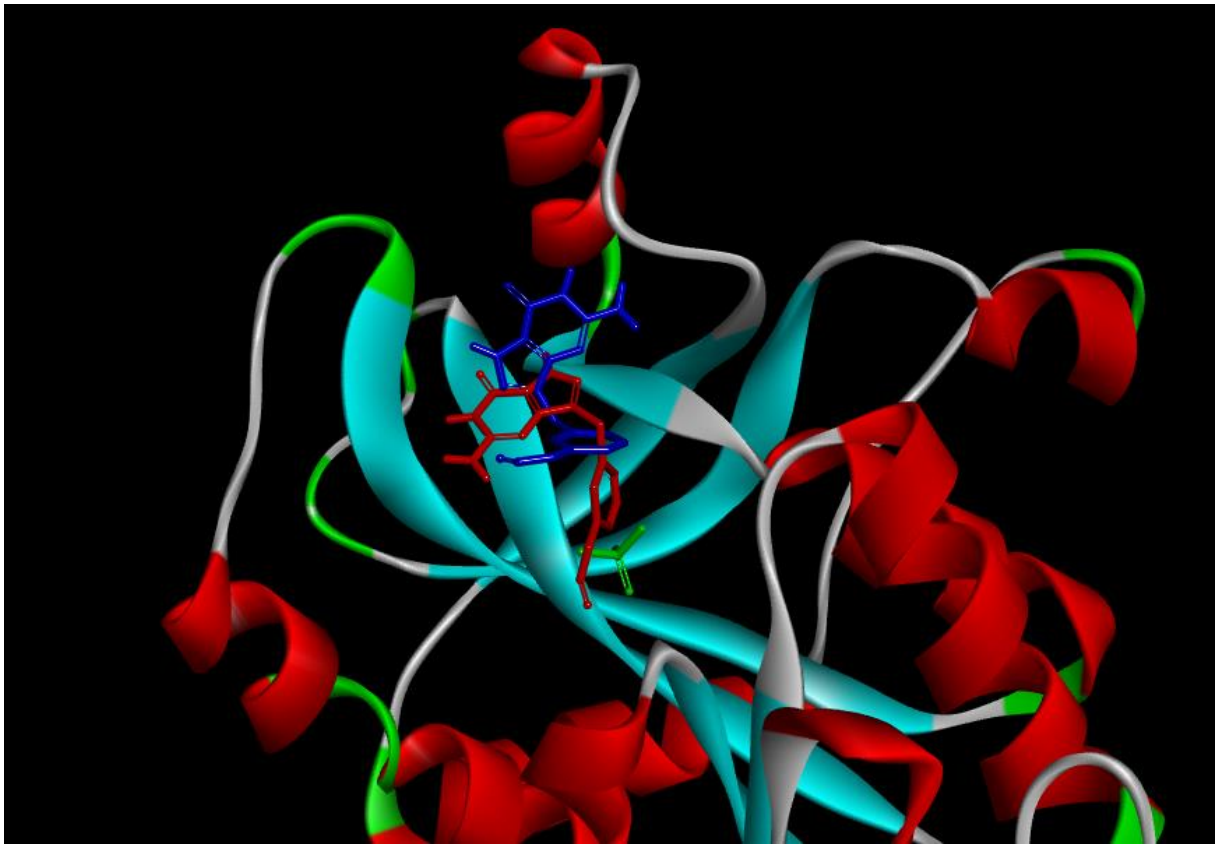
Comparison of ligand number 23



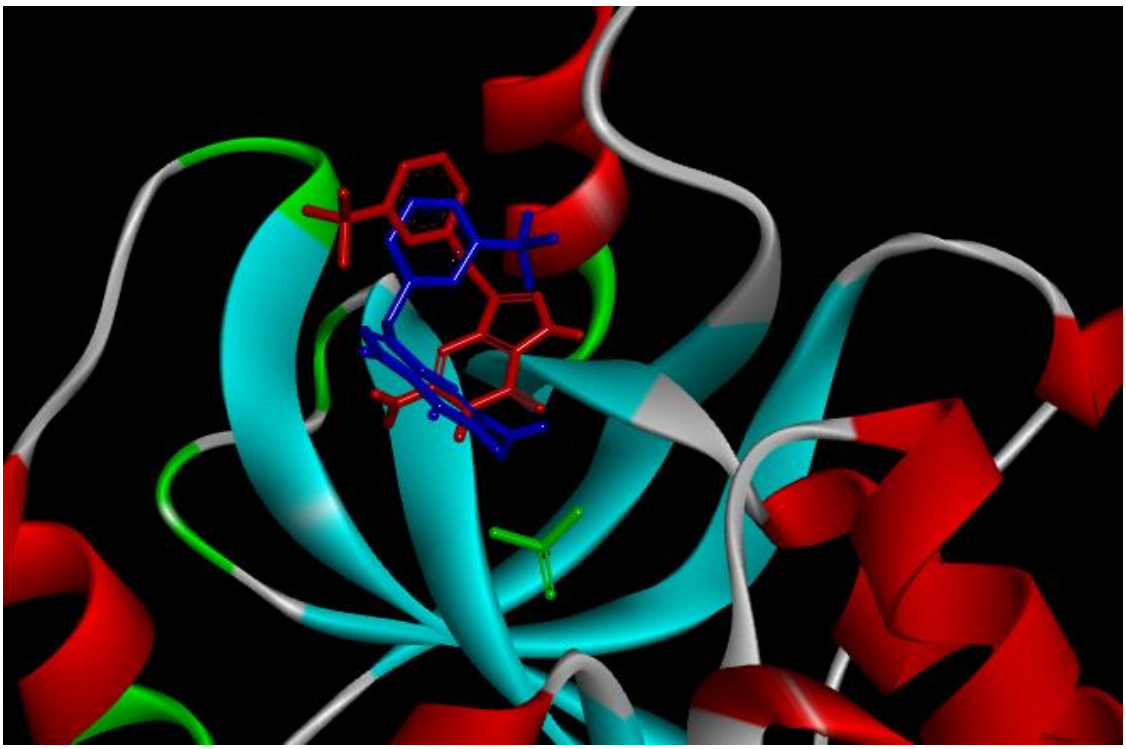
Comparison of ligand number 24



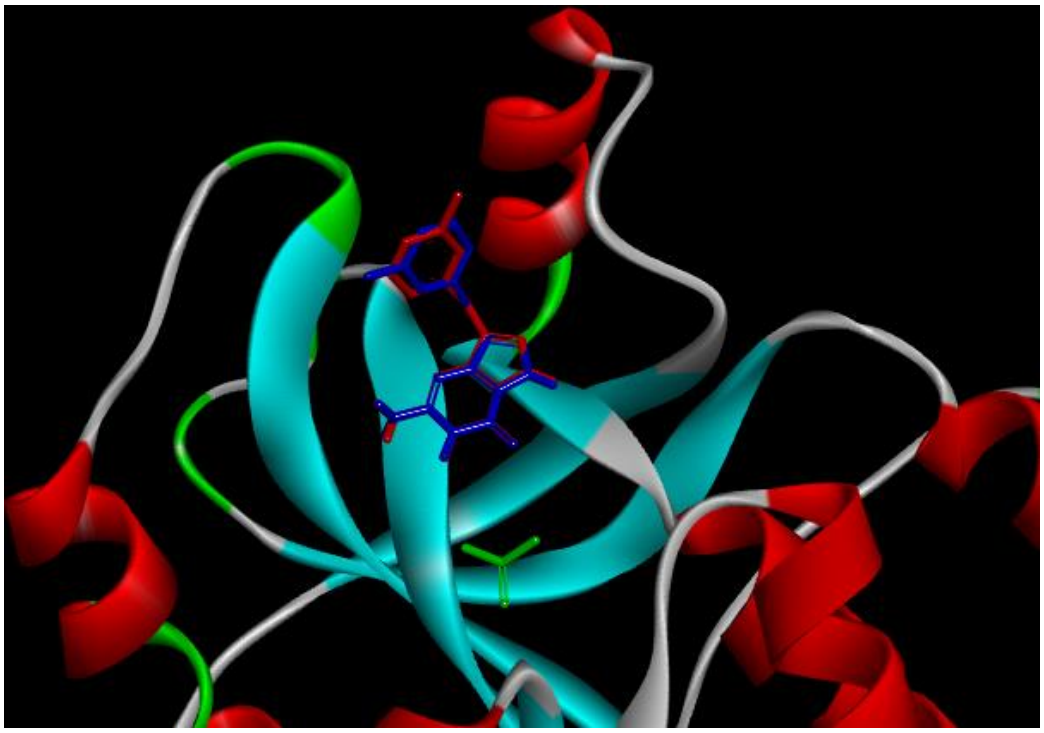
Comparison of ligand number 27



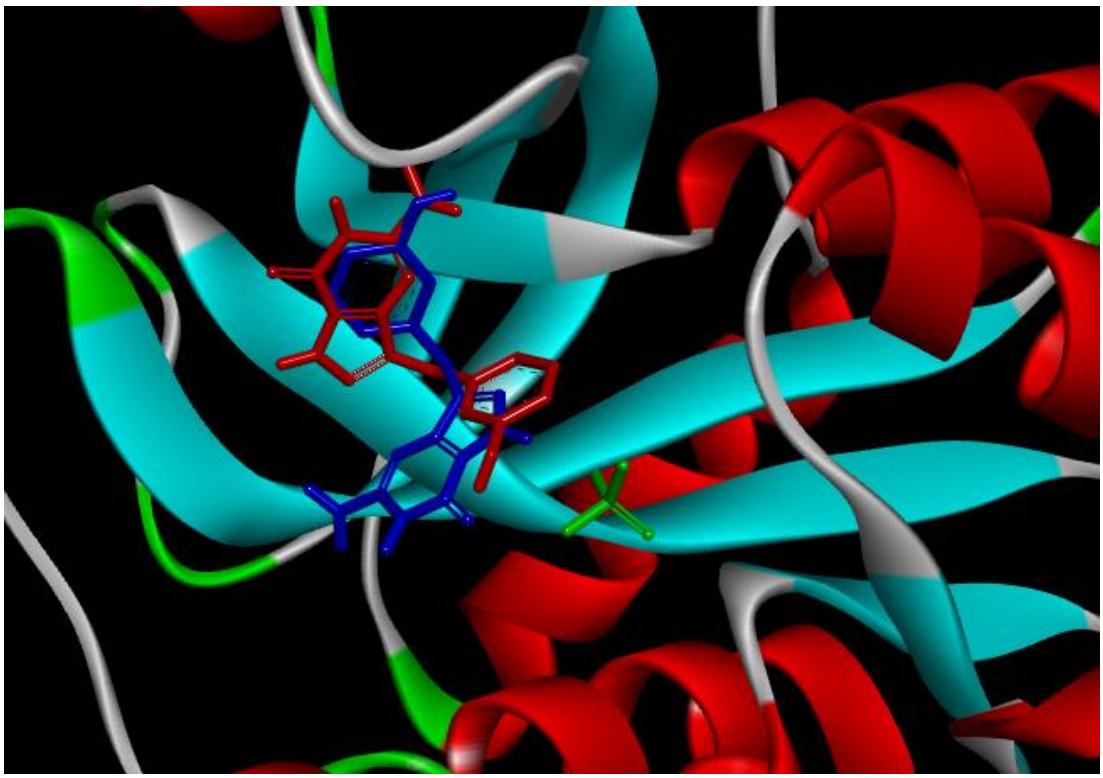
Comparison of ligand number 28



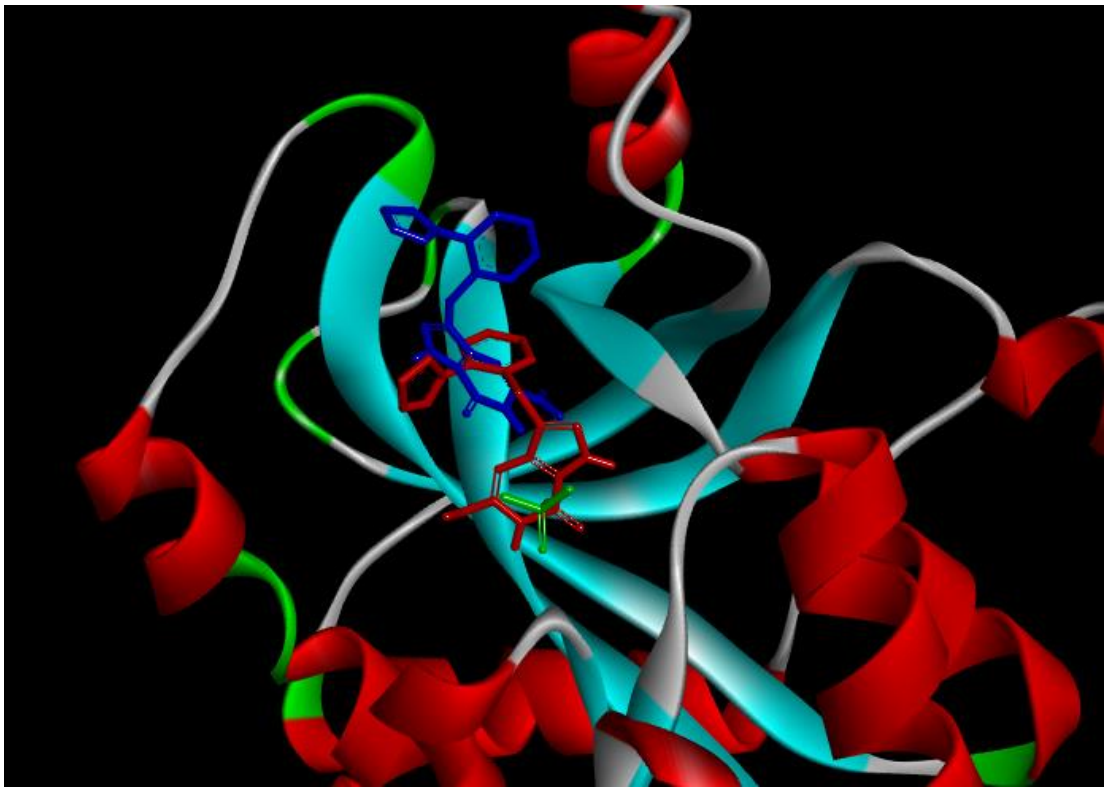
Comparison of ligand number 30



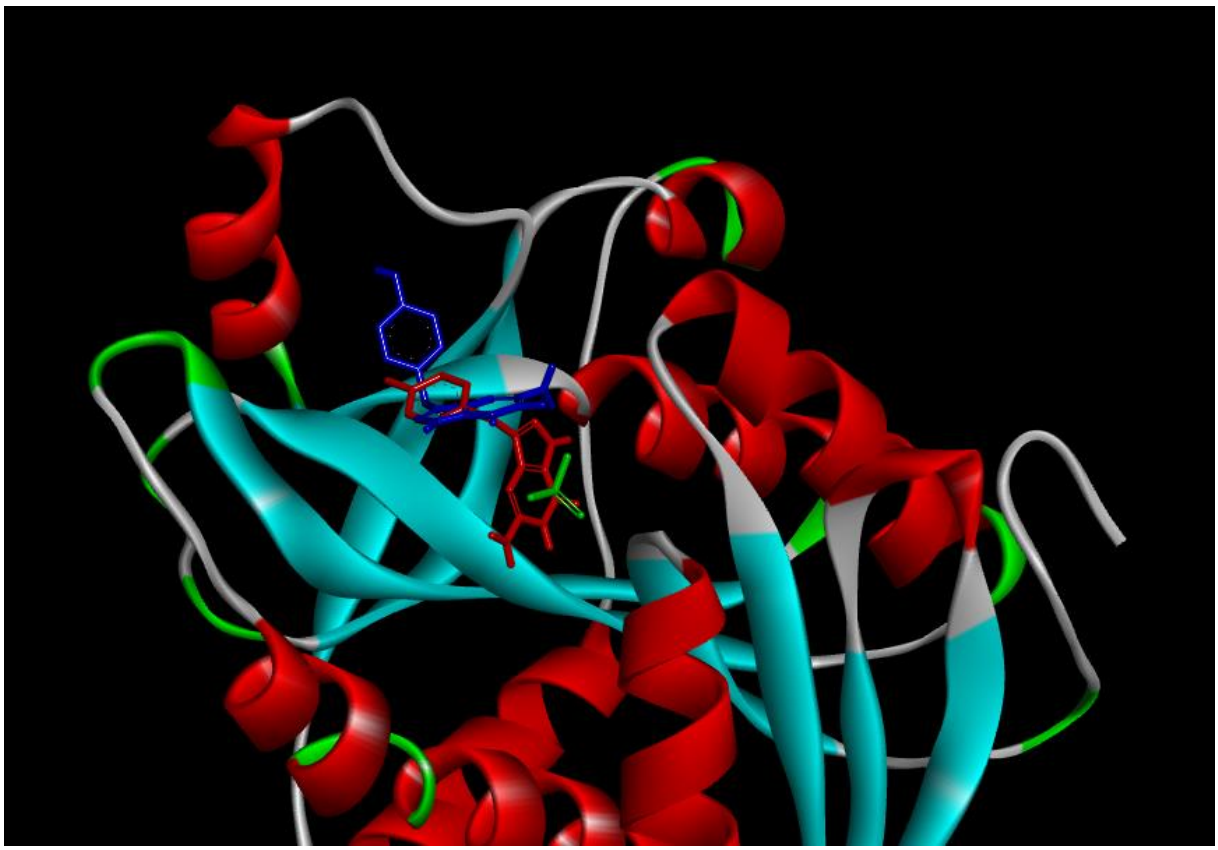
Comparison of ligand number 31



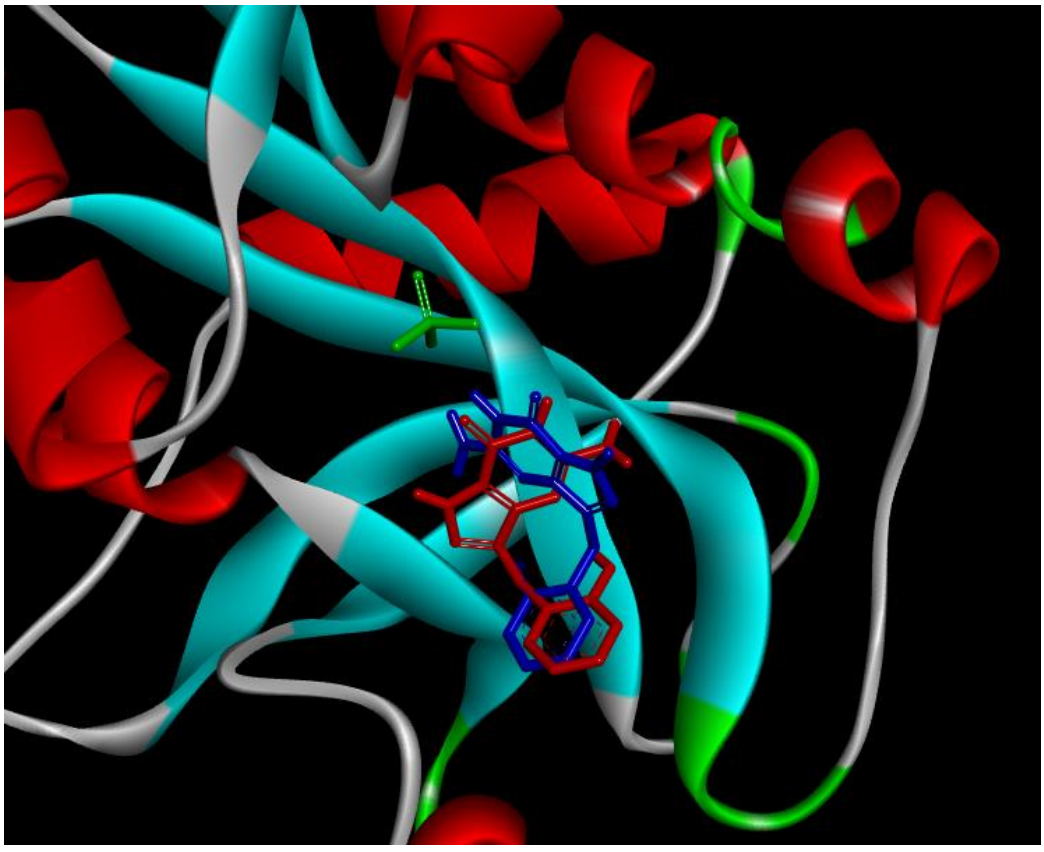
Comparison of ligand number 33



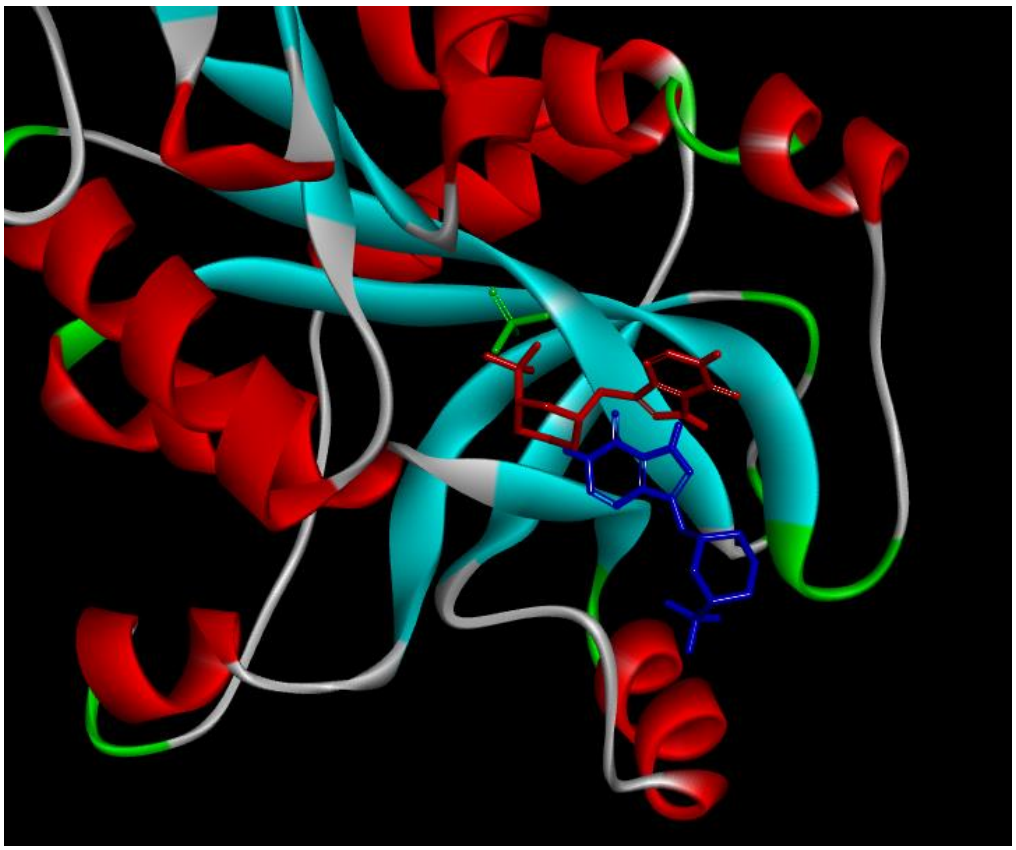
Comparison of ligand number 37



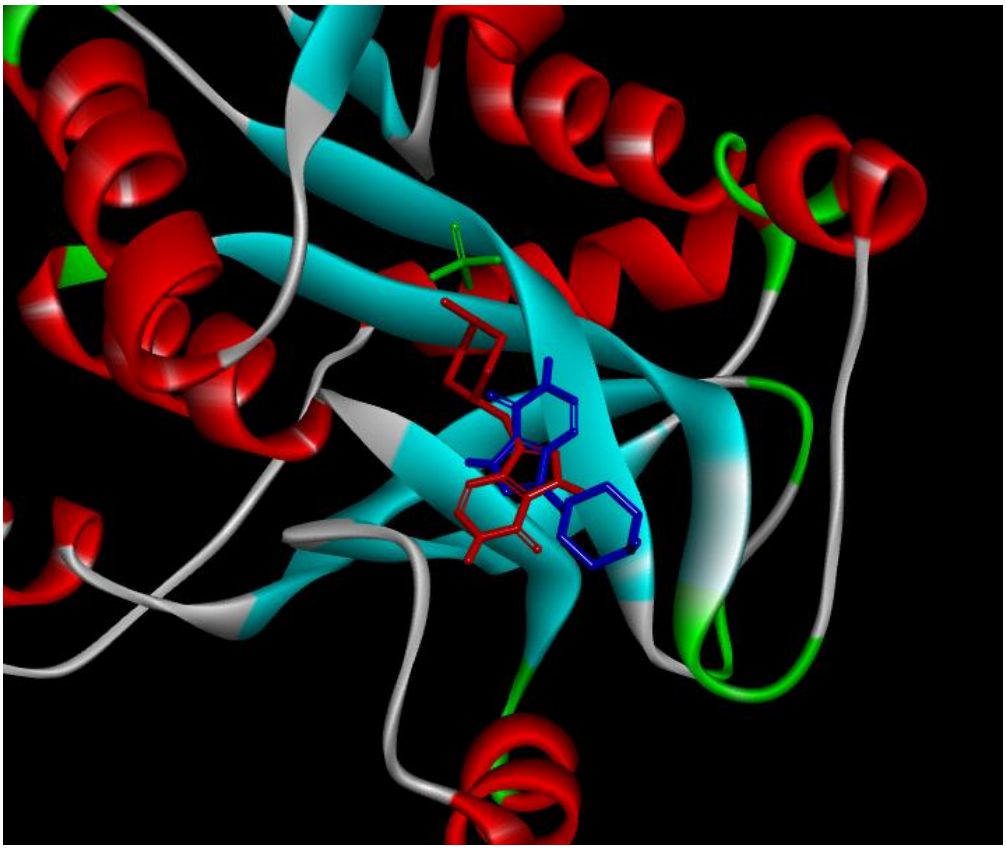
Comparison of ligand number 38



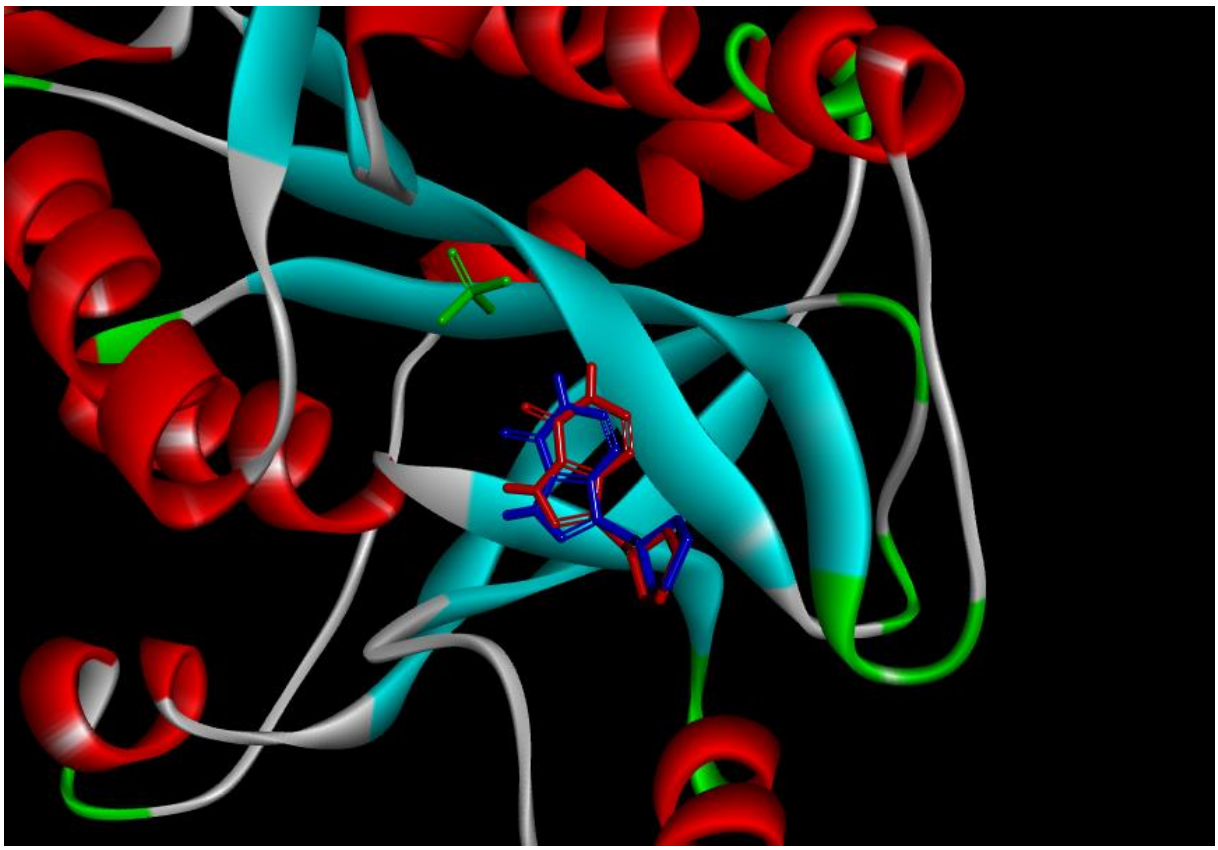
Comparison of ligand number 41



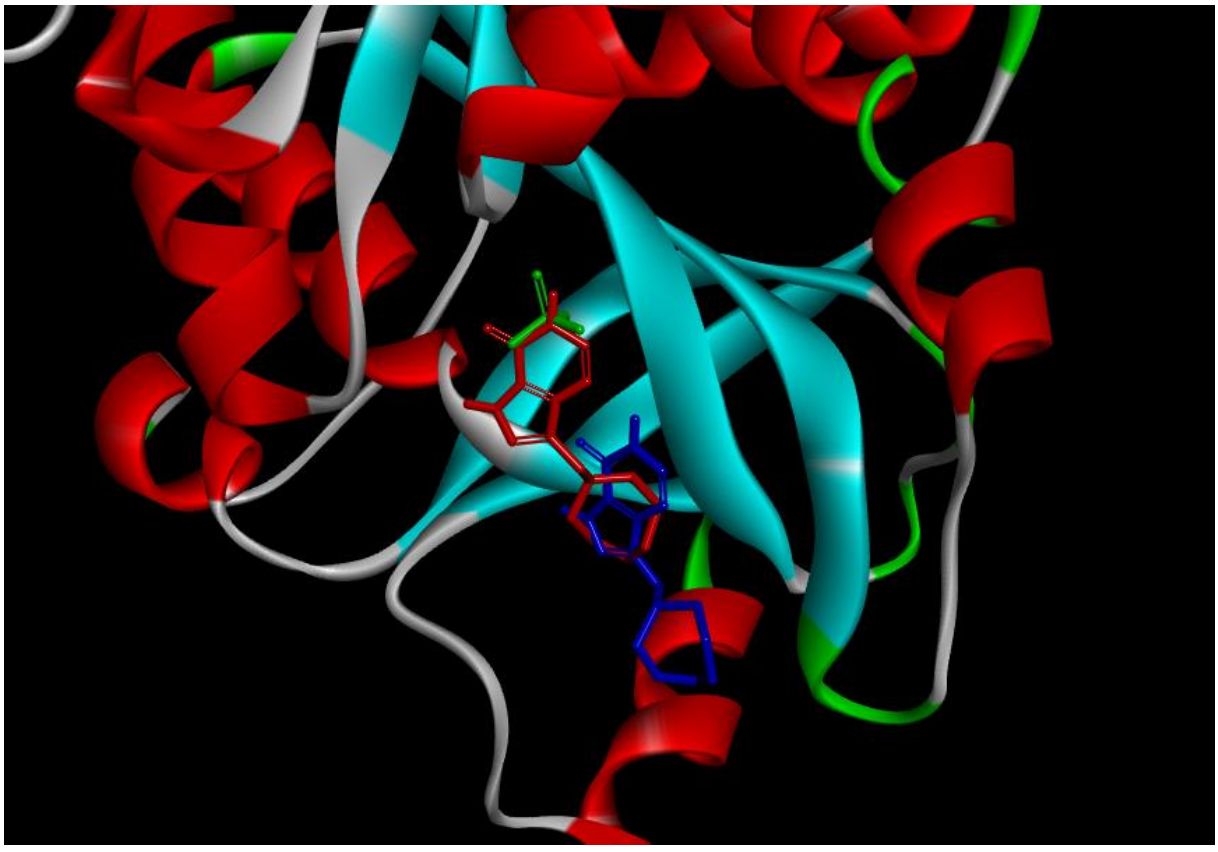
Comparison of ligand number 42



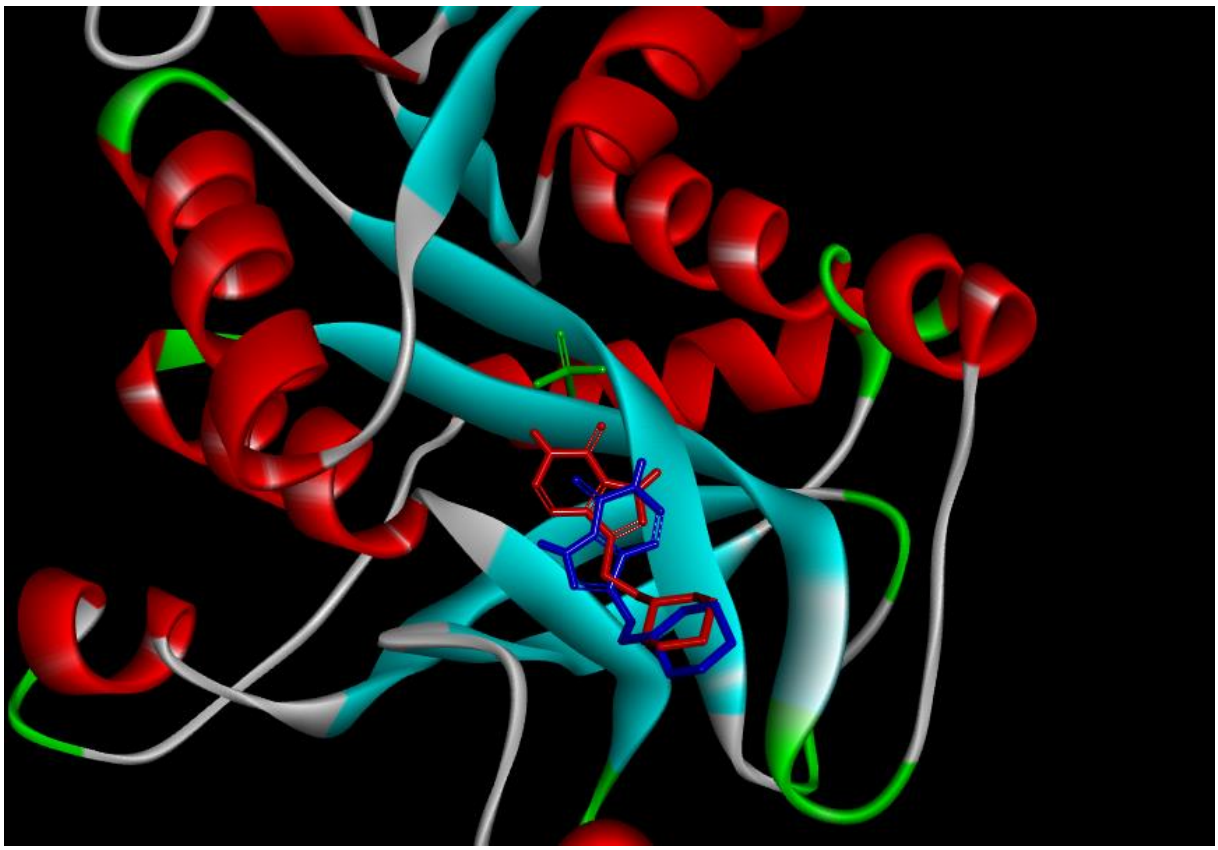
Comparison of ligand number 43



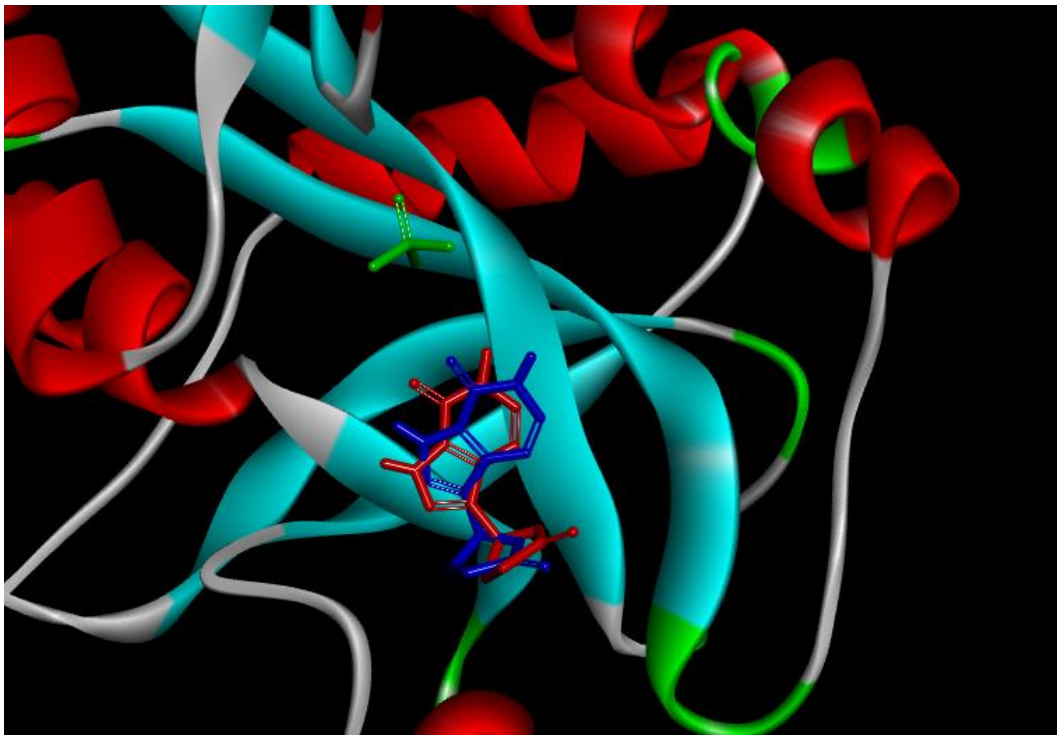
Comparison of ligand number 44



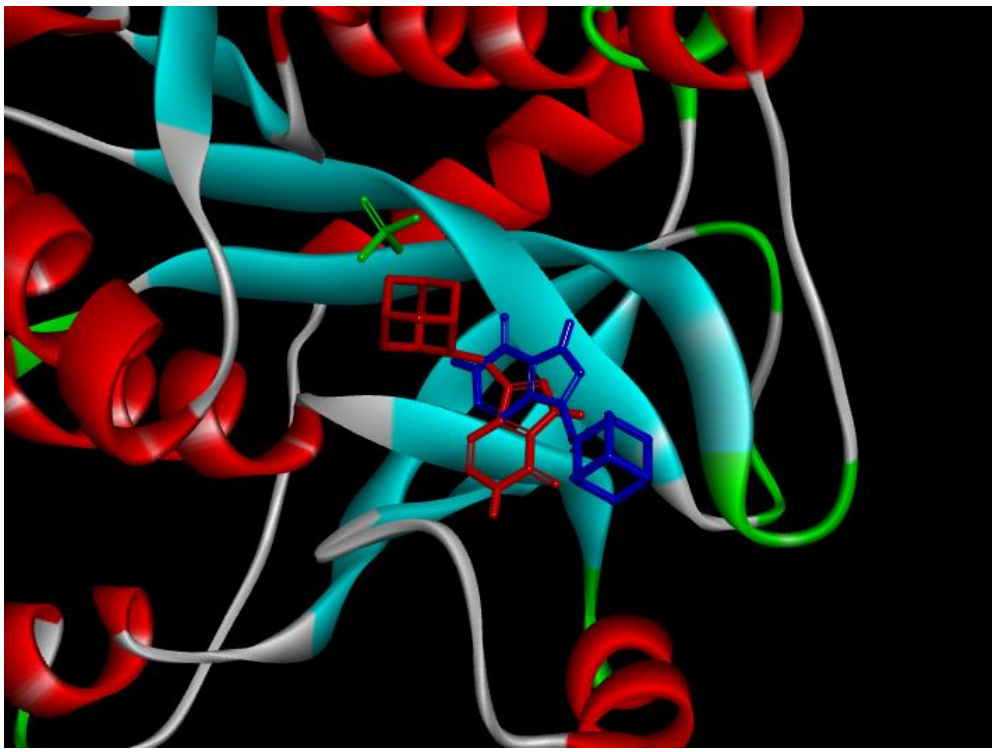
Comparison of ligand number 45



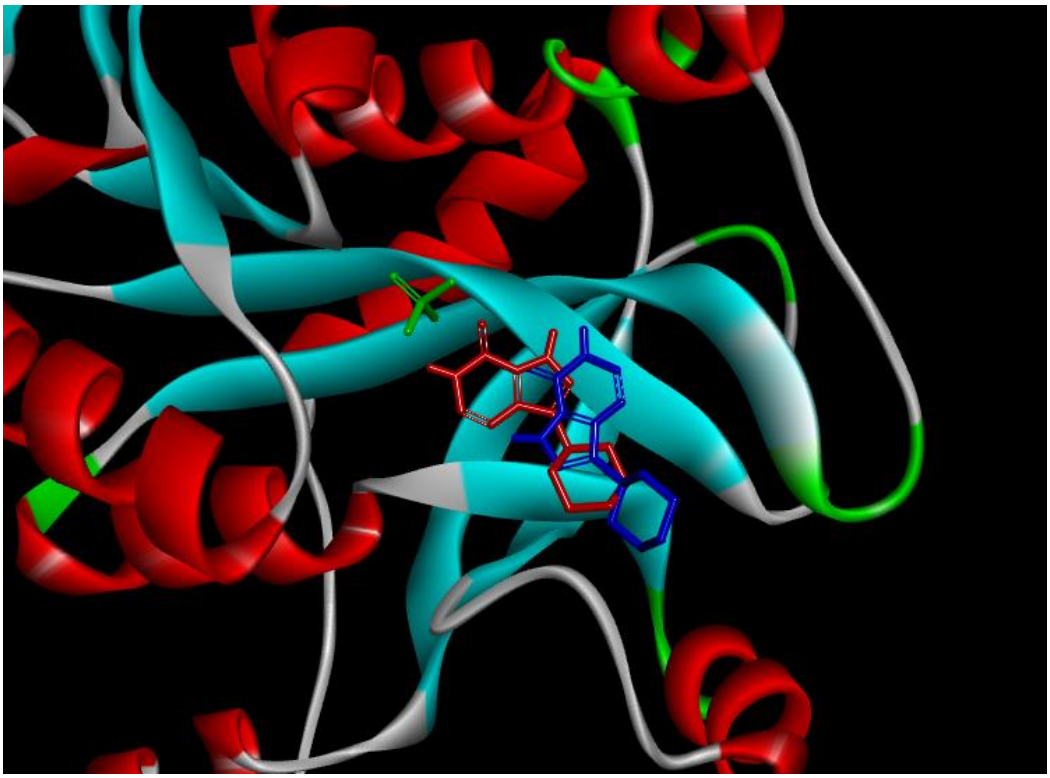
Comparison of ligand number 46



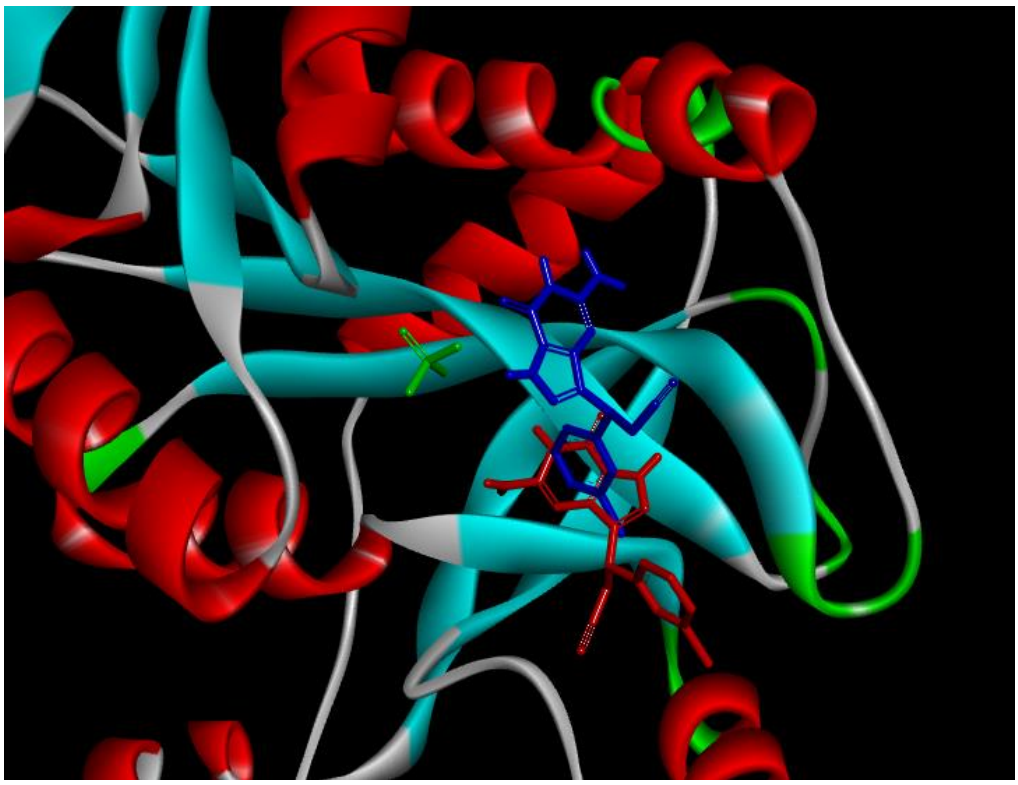
Comparison of ligand number 47



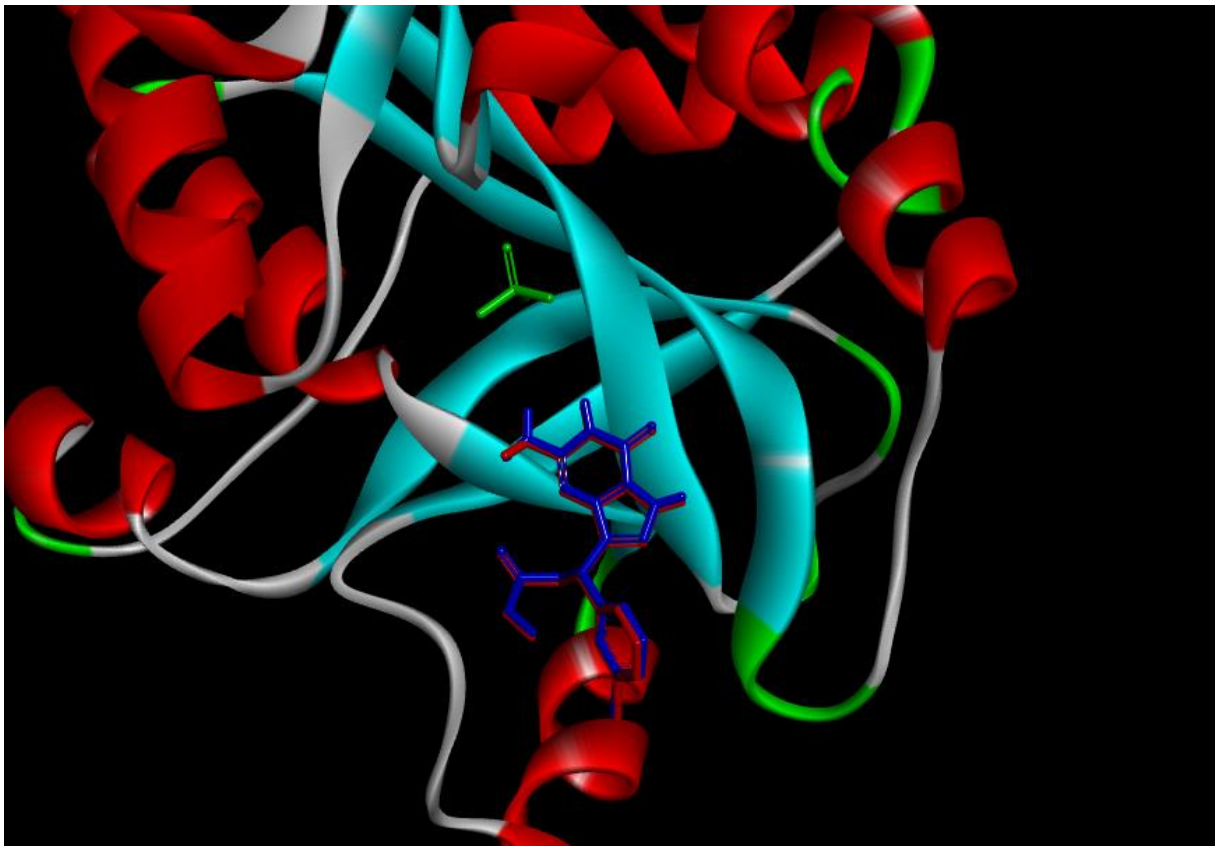
Comparison of ligand number 49



Comparison of ligand number 51



Comparison of ligand number 53



Biography

Mr. Panuwat Viriyaparadon was graduated from Thai Christian School in Bangkok, Thailand. Currently, he is studying in fourth year for Bachelor of Science Applied Chemistry (BSAC) program majoring in Industrial Chemistry and Management, the Department of Chemistry, Faculty of Science, Chulalongkorn University. Contract: panu.viri.inrod00@gmail.com

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