

MOLECULAR MODELLING AND QUANTUM CHEMICAL CALCULATIONS STUDY ON ANTIFOLATE ANTIMALARIAL INHIBITORS

INTRODUCTION

Malaria Disease

Malaria is as one of the deadliest diseases around the world. Annually, this disease affects 500 million people and causes 2.5 million deaths. Malaria disease is caused by protozoa parasites. It was discovered by Charles Louis Alphonse Laveran, a French army surgeon stationed in Constantine, Algeria as shown his picture in Figure 1. In 1907, Laveran was awarded the Nobel Prize for this discovery (www.malariasite.com, Warhurst, 2002).

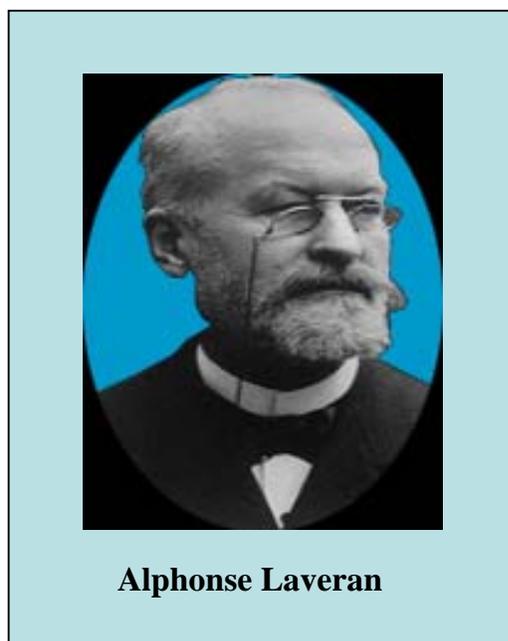


Figure 1 Alphonse Laveran was the first to notice parasites in the blood of a patient suffering from malaria.

Next, Ronald Ross (see in Figure 2), a British officer in the Indian Medical Service, discovered that malaria is transmitted from one person to another by mosquitoes. Based on his discovery, not only the problem of malaria transmission was solved but also he was awarded the Nobel Prize in 1902 (www.malariasite.com, Warhurst, 2002).

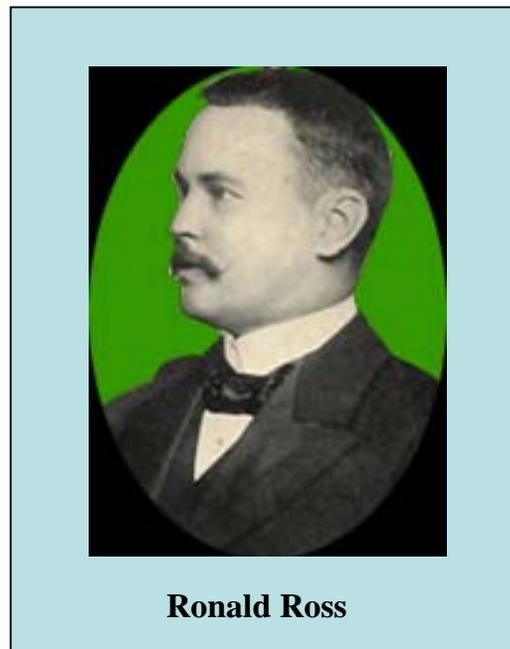


Figure 2 Ronald Ross was the first to demonstrate that a mosquito could transmit a malaria parasite.

Malaria is a protozoan parasitic disease which can be transmitted to humans by species of female Anopheline mosquitoes. There are four species; *Plasmodium falciparum* (*Pf*), *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* which can produce malaria disease. Malaria caused by *Pf* is the most critical form (Botelho, 1971, Warhurst, 2002, Collins and Jeffery, 2007). The life Cycle of *Pf* is divided into three majority stages consisting Human Liver Stages or Exo-erythrocytic Cycle, Human Blood Stages or Erythrocytic Cycle and Mosquito Stages or Sporogonic Cycle.

Dihydrofolate Reductase Enzymes

In this study, we have focused on the dihydrofolate reductase enzyme of *Pf* (*Pf*DHFR). The *Pf*DHFR plays an important role in the folate metabolism. It is an essential enzyme for maintenance of adequate levels of tetrahydrofolate (THF), which is fundamental in the metabolic cycle of biosynthesis of deoxythymidylate or deoxythymidine 5'-monophosphate (dTMP) as shown in Figure 3. In this cycle, thymidylate synthase (TS) catalyzes the conversion of deoxyuridine 5'-monophosphate (dUMP) and N^{5,10}-methylene tetrahydrofolate to dTMP and dihydrofolate (DHF), respectively. DHFR catalyzes the subsequent reduction of DHF to THF. Finally, serine hydroxymethyltransferase (SHMT) catalyzes the regeneration of N^{5,10}-methylene tetrahydrofolate, which guarantees the continuation of dTMP biosynthesis. Hence, the inhibition of *Pf*DHFR or of any other enzyme in the cycle prevents the formation of new dTMP molecules, leading to the interruption of deoxyribonucleic acid (DNA) synthesis. Consequently, the malarial parasite is death (Delfino, *et al.*, 2002, Nzil, 2006, Yuthavong, 2002, Yuthavong, *et al.*, 2006).

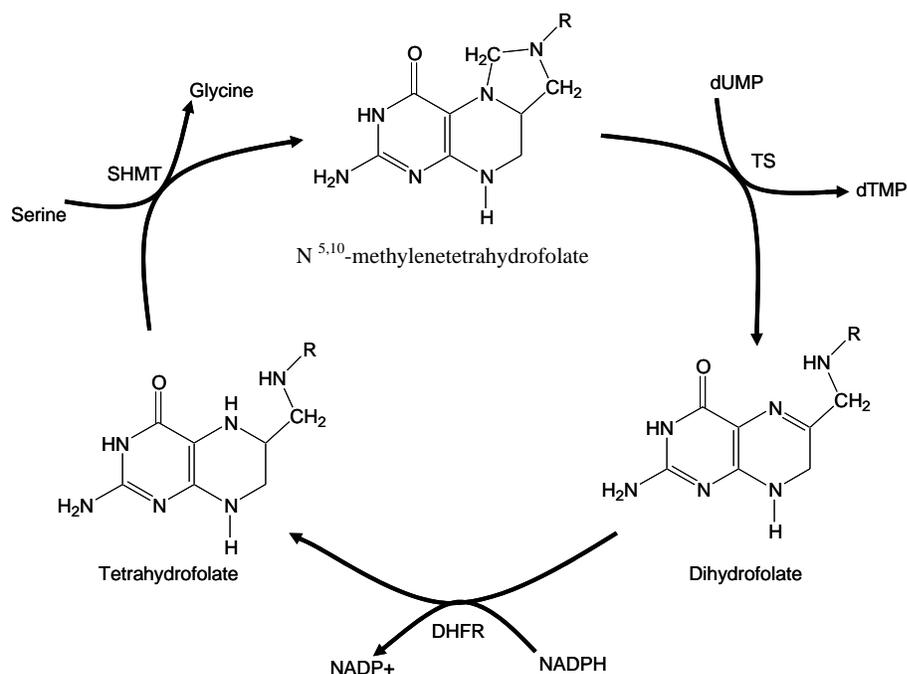


Figure 3 Biosynthetic Cycle of dTMP (R = pABA + L-glutamate)

Mutation of *Pf*DHFR

Soon after pyrimethamine, an antifolate antimalarial was introduced for treatment of malaria; malaria became resistant to the drug. It was found that the resistance was due to mutation of *Pf*DHFR enzyme. The mutation lowers drug binding affinity of the enzyme. Two series of *Pf*DHFR mutants were identified, being Ser108Thr and Ser108Asn present as single and multiple mutations with other residues. Ala16Val+Ser108Thr is the sole member of the Ala16Val series while Ser108Asn series comprises of several naturally occurring forms of multiple mutations. It was proposed that the multiple mutations stemmed from a single mutation of Ser108 to Ser108Asn was followed by stepwise mutations of Cys59Arg, Asn51Ile and Ile164Leu to yield double (Asn51Ile+Ser108Asn and Cys59Arg+Ser108Asn), triple (Asn51Ile+Cys59Arg+Ser108Asn and Cys59Arg+Ser108Asn+Ile164Leu) and quadruple mutations (Asn51Ile+Cys59Arg+Ser108Asn+Ile164Leu) as shown by diagram in Figure 4 (ref). The quadruple mutant is the most resistant form found to date.

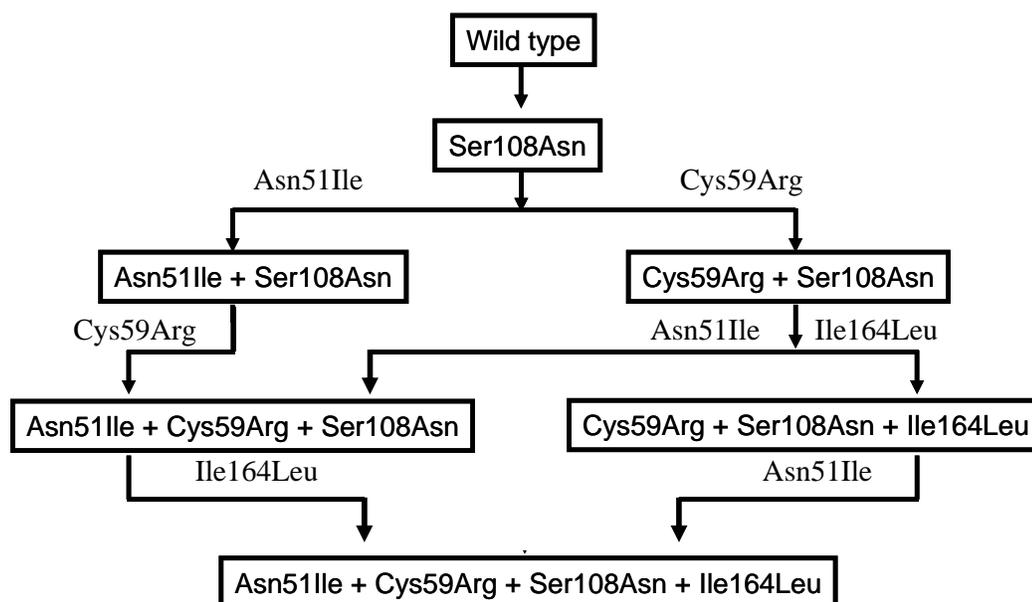


Figure 4 The proposed evolution of Ser108Asn series of *Pf*DHFR mutants

Antifolate Antimalarial Inhibitors

During the Second World War, proguanil was the first reported antimalarial antifolate agent and was synthesized by Imperial Chemical Industries. Proguanil is a prodrug which is metabolized to its active triazine form cycloguanil (Cyc), an inhibitor of the parasite DHFR (Schmidt, 1969 and Meshnick, *et al.*, 2001). This drug can be used alone or in combination with chloroquine, dapsone and atovaquone (Polhemus, *et al.*, 2008, Nakato, *et al.*, 2007, Krudsood, *et al.*, 2005, 2007, Matsika, *et al.*, 2006, Nosten, *et al.*, 2006, Camus, *et al.*, 2004, Muehlen, *et al.*, 2004, Fidock, *et al.*, 1998, Yeo, *et al.*, 1997, Edstein, *et al.* 1997). Although, proguanil has given a good inhibitory for wild type *Pf*DHFR, its activity is poor against the mutant enzymes. Thus, the potency of proguanil had led to the development of new potent analogues which demonstrated the pattern of antimalarial potency increasing with increased chlorination of the phenyl ring and the increased length of linker between the phenyl ring and the diaminopyrimidine ring. These studies led to the discovery of chlorproguanil, clociguanil (BRL 50216) and BRL 6231 (WR99210) (Rieckmann, *et al.*, 1996) as shown in Figure 5.

BRL 6231 or WR99210 has three chlorine atoms on the phenyl ring and a long aliphatic linker (Figure 5). WR99210 is a good inhibitor against wild type *Pf*DHFR and those with multiple mutations *in vitro*. However, its efficacy in animal models is poor due to low bioavailability of WR99210 thus preventing its development as an antimalarial agent. (Rieckmann, *et al.*, 1996, Hekmat-Nejad and Rathed, 1997 and Delfino, *et al.* 2002).

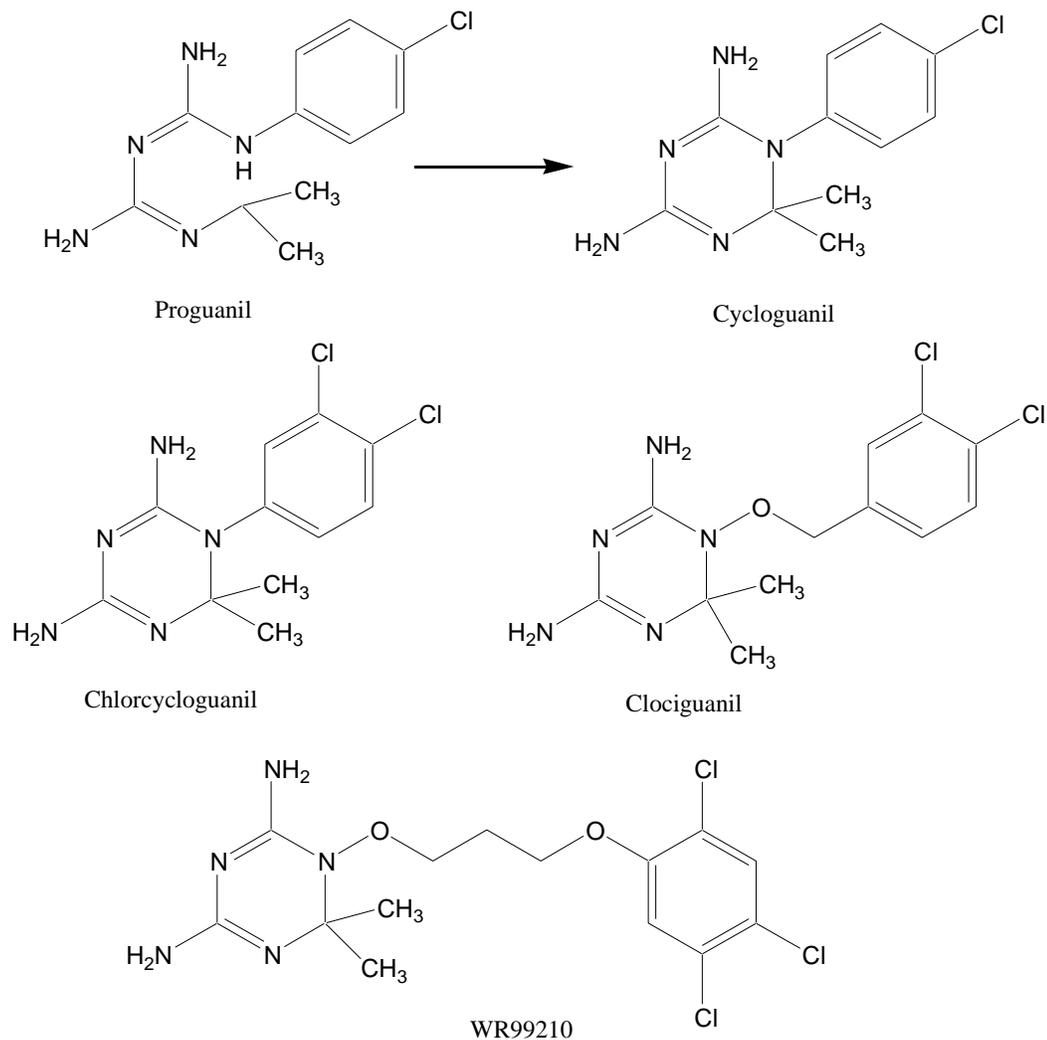


Figure 5 Chemical structures of proguanil and its derivatives

Pyrimethamine (Pyr) belongs to the 2,4-diaminopyrimidine derivative family. Its structure is as shown in Figure 6. Since the structures of proguanil is similar to 2,4-diaminopyrimidines, it was hypothesized that 2,4-diaminopyrimidine analogs could have antimalarial activity. Screening of antimalarial activity of 2,4-diaminopyrimidine analogs had led to the identification of Pyr. Pyr has been the most widely used antimalarial antifolate agent so far. It is used in combination with sulfadoxine or sulfalene, and to a lesser extent it has been used in monotherapy, also known as

Daraprim (Saadat *et al.*, 2005, Alker, *et al.*, 2004, Alam, *et al.*, 2007, Gatton, *et al.*, 2006, Zongo, *et al.*, 2005).

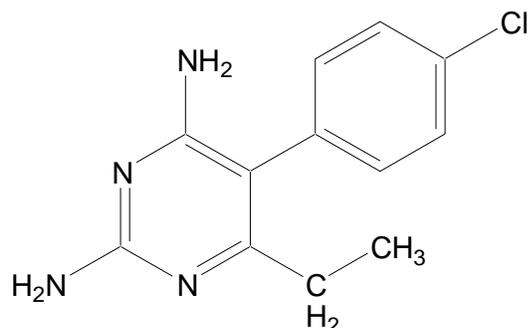


Figure 6 Chemical structures of Pyrimethamine

Unfortunately, the resistances of *Pf*DHFR to the Cyc and Pyr drugs have rapidly reduced their clinical utility. It has been reported that antifolate resistances are consistent with point mutations in *Pf*DHFR started from Ser108 to Asn108. Additional point mutations of Asn51Ile, Cys59Arg and Ile164Leu were associated with higher levels of resistance (Uhlemann, *et al.*, 2005, Chusacultanachai, *et al.*, 2002, Gregson and Plowe, 2005, Nzila, 2006, Yuthavong, 2002, Vilaivan, *et al.*, 2003, Sirawaraporn, *et al.*, 1997, 2002, Peterson, *et al.*, 1990, Sirichaiwat, *et al.*, 2004, Yuthavong, *et al.*, 2000, 2005). These have led to an urgent need for new effective antifolate antimalarials against the resistant type *Pf*DHFRs. For example, in 2004, Kamchonwongpaisan and co-workers reported series of Pyr and the Cyc antifolate derivatives with their inhibition constants (K_i) in both the wild type and the multiple mutant *Pf*DHFRs (Kamchonwongpaisan, *et al.* 2004)). This research has led to further work on analysis of the relationship between structure and the activity and the development of new and effective antifolate antimalarials against in *Pf*DHFRs.

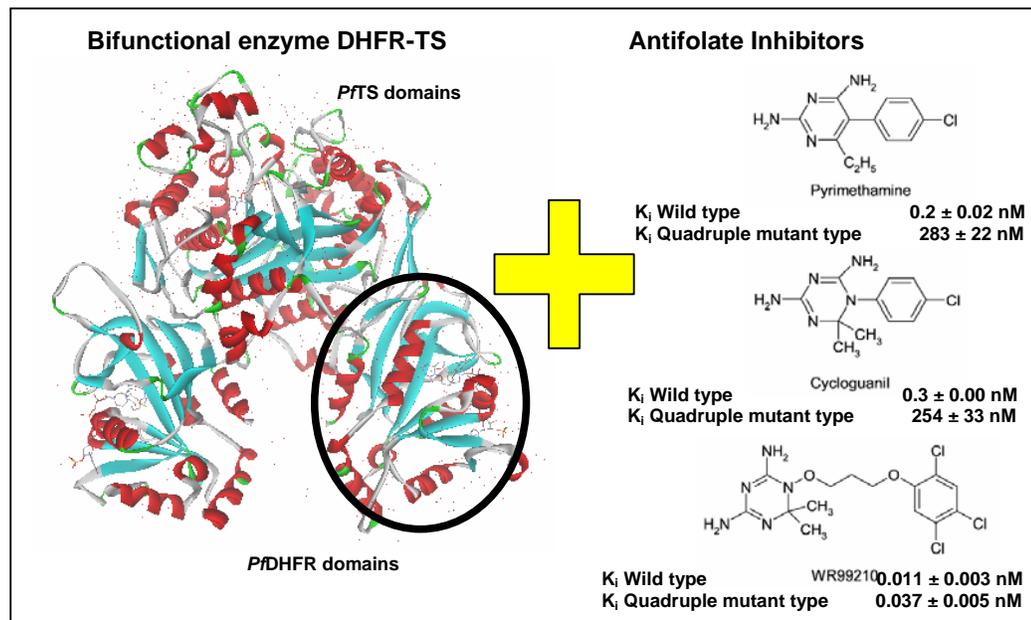


Figure 7 Structures of DHFR-TS and binding affinity of Pyr, Cyc and WR99210 antifolates against wild type and quadruple mutant (Asn51Ile+Cys59Arg+Ser108Asn+Ile164Leu) of *PfDHFRs*

Three-dimensional Quantitative Structure-activity Relationship and Its Applications

Several techniques and approaches of computer-aided drug design have been used in drug discovery, design and development. One of the popular approaches is Quantitative Structure–Activity Relationship (QSAR) studies (Hansch and Fujita, 1964, Hansch and Leo, 1995). QSAR is extensively used as an emerging cheminformatics tool for information retrieval to design and develop new drug candidates and interpret the binding pattern for ligand-receptor interactions.

The three-dimensional quantitative structure activity relationship (3D-QSAR) models are constructed by correlating the 3D fields to the corresponding experimental activities of ligands with respect to a common target receptor. As 3D molecular modeling becomes widely available to workers in structural biology, properties

calculated from atomic coordinates are used increasingly to explain and predict biological activity (Brown and Martin, 1997). Molecular modeling can produce any desired number of explanatory descriptors for each structure—far more than the number of activity data to be explained. There are many techniques for 3D-QSAR, for example, Comparative Molecular Field Analysis (CoMFA) bases its predictions upon field values calculated at each point of a 3D grid around the molecular structures. The field values are highly correlated, having been derived from the molecular descriptors such as atomic charges and positions (Cramer, *et al.*, 1988). In model calculations, the linear regression by partial least squares (PLS) is used for producing a formula which fits the training data (Clark and Cramer, 1993, Bush and Nachbar, 1993). The advantage of PLS method is reducing the explanatory data to a small number of components. Within the molecular modeling package SYBYL, PLS is the recommended regression method for analysis of CoMFA fields. The advantages of CoMFA are the ability to predict the biological activities of the molecules and to represent the relationships between steric/electrostatic properties, calculated according to Lennard-Jones and Coulomb potentials, respectively, and also the biological activity in the form of contour maps to provide the key features of both the ligand-receptor interaction and the topology of the receptor. The CoMFA results can be used for guiding the new design potent compounds, based on the same template constructions.

Quantum Chemical Calculations

As theoretical/computational chemistry has gained a major role in studies of chemical problems in the last few decades, it became a challenge for theoreticians to accurately treat large molecular systems such as ligand-enzyme in biochemistry. The quantum computational chemistry calculations can be used to investigate inhibitor-enzyme interactions at the molecular level. The obtained strong repulsive energy of some amino acids that was quantified implies that this is the caused of the antifolate resistance. On the other hand, the strong interactions between some amino acids and the inhibitor should be investigated to discover further new designs. Therefore, an understanding of the particular interaction energy of individual amino acids with the

antifolate inhibitors at molecular level is required to support the identification of these new structural modifications.

Not only the term of interaction energy can be calculated, the binding energy can also be extrapolated. For binding energy calculations of biomolecular systems, no single quantum chemical calculation can be performed. Therefore the use of the hybrid methods is powerful for enzyme-ligand interactions, which mimic of both accuracy and computational cost. One of the popular hybrid methods known as ONIOM (our Own N-layer Integrated molecular Orbital molecular Mechanics) developed by Prof. Keiji Morokuma group (Dapprich, *et al.*, 1999). This method can be capable to describe the molecular details of ligand-enzyme binding interaction in many interested targets. In the ONIOM approach, a large molecular system can be partitioned into multilayer regions. The region of the system where the chemical process takes place is treated with an appropriately accurate method, while the remainder of the system is treated at a lower level. Therefore, the ONIOM method can be performed on ligand-enzyme systems to extrapolate the binding energy (Kuno, *et al.*, 2003, 2006, Matsubara, *et al.*, 2007, Saen-oon, *et al.*, 2005, 2007)

Objectives

In the present work, ligand-based drug design approaches using CoMFA has been applied to the class of Pyr and Cyc derivatives with the aims of:

1. To find a mathematical model suitable for analysis of structure and biological activity of Pyr and Cyc derivatives which are active against wild type and quadruple mutant (Asn51Ile+Cys59Arg+Ser108Asn+Ile164Leu) type of *Pf*DHFR enzymes.
2. To give an insight in the guiding for design of potent Pyr and Cyc inhibitors.

In addition, structure-based drug design approaches using quantum chemical calculations and three-layer ONIOM methods have been performed on the complex of the three types of antifolates (Pyr, Cyc and WR99210) and *Pf*DHFR enzymes with the aims of:

3. To investigate the particular interaction energies between antifolate inhibitors and amino acids surrounding the binding pocket of *Pf*DHFR enzymes.

3.1 To compare the inhibitors which bound in quadruple mutant type *Pf*DHFR using Pyr as represented a poor inhibitor and Pyr6 for potent inhibitor.

3.2 To compare the *Pf*DHFR enzymes which complexed with Cyc (or WR99210) drug focusing on the wild type and the quadruple mutant type *Pf*DHFRs.

4. To calculate the binding energies of the systems which were defined Pyr, Cyc, and WR99210 antifolates as the liganded complex of quadruple mutant *Pf*DHFR.