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Mutational Effect to Particular Interaction Energy of Cycloguanil Drug to Plasmodium Plasmodium Falciparum Dihydrofolate Reductase Enzymes

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***Abstract*—**In order to find the particular interaction energy between cylcloguanil and the amino acids surrounding the pocket of wild type and quadruple mutant type *Pf*DHFR enzymes, the MP2 method with basis set 6-31G(d,p) level of calculations was performed. The obtained interaction energies found that Asp54 has the strongest interaction energy to both wild type and mutant type of -12.439 and -11.250 kcal/mol, respectively and three amino acids; Asp54, Ile164 and Ile14 formed the H-bonding with cycloguanil drug. Importantly, the mutation at Ser108Asn was the key important of cycloguanil resistant with showing repulsive interaction energy.

***Keywords*—**Cycloguanil, DHFR, malaria disease, interaction energy, quantum calculations

I. Introduction

EVEN nowadays a malaria disease has been decreasingly infected but the drug development of such kind of the disease still moves forward both experimental and theoretical parts. The majority study focuses on the cause of the disease, *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) which is an important and well-defined target for malaria chemotherapy [1], [2]. This enzyme is a bifunctional enzyme with Thymidilate synthase (TS) [3]. Both are essential for producing a precursor for DNA biosynthesis. Inhibition of DNA synthesis through inhibition of *Pf*DHFR by Pyrimethamine (Pyr), Cycloguanil (Cyc) and other antifolates leads to parasite death [4]-[9]. The Cyc is one of an effective antifolate in wild type malarial chemotherapy and is studied in this work. Unfortunately, Cyc role in clinical antimalarial chemotherapy has been worsened by emergence of parasite resistant to drug [10], [11]. Especially, a quadruple mutant type of *Pf*DHFR (Asn51Ile, Cys59Arg, Ser108Asn and Ile164Leu) is associated with the highest level Cyc resistances with reduced inhibition constants (K_i) of 254 nM, approximately 846-times of wild type enzyme [12]-[16].

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Therefore, an application of quantum chemical calculations for investigating the particular interaction between amino acids surrounding the binding pocket and the drug Cyc is very useful to understand the molecular details the drug to the wild type and quadruple mutant enzymes [17]-[20].

II. Computational Details

Based on no an available x-ray structure of the Cyc and *P*fDHFR enzymes, so, the crystal structures of Cyc derivative, WR99210, which was bounded into the *Pf*DHFR both wild type (PDB code 1J3I) and quadruple mutant type (PDB code 1J3K) were used to be the starting geometry of the Cyc/*Pf*DHFRs complexes. Then the AMBER molecular mechanic minimization was firstly performed to optimize the complex structures. The Cyc structure was fully optimized at the B3LYP/6-31G(*) level of calculations, and then the RESP electrostatic charges of all atoms in the Cyc inhibitor were also calculated using the Gaussian 03 package. The resultant structural and electrostatic charges were used to prepare the molecular mechanical Amber force field parameters using the Antech Amber module. For NADPH cofactor AMBER parameters were taken from AMBER Parameter Database. The TIP3PBOX water molecules were used to solvate both complexes. Four chloride ions were added to neutralize the system. The minimization process of the complex structures was performed to eradicate bad contacts and to relax the complex models. A cutoff distance of 12 Å was set for the non-bonded pair interactions. All molecular mechanical minimizations were carried out using the AMBER 9.0 simulation package. The obtained molecular mechanical structures of the wild type and the quadruple mutant type *Pf*DHFRs complexed with the Cyc were used as the starting geometry of the particular interaction energy calculations at the MP2/6-31G(d,p) level for the Cyc inhibitor with respect to both target enzymes. All residues located with at least one atom interacting with any atoms of the inhibitor within the interatomic distance of 4 Å were selected to calculate the interaction energy with the inhibitor based on quantum chemical calculations. The twenty selected residues were Ile14, Cys15, Ala16, Val45, Leu46, Trp48, Cys50, Asn51Ile, Asp54, Met55, Tyr57, Phe58, Met104, Ser108Asn, Ser111, Ile112, Leu119, Ile164Leu,

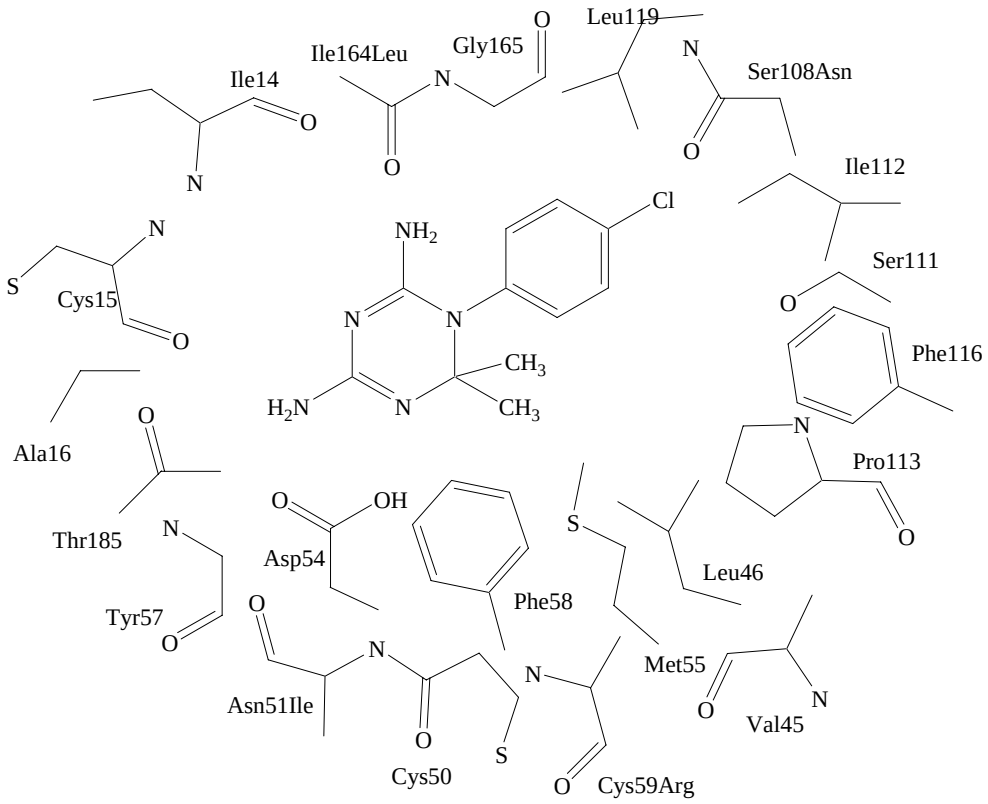

Fig. 1 The 2D scheme of the adopted model system of Cyc bound to the wild type and quadruple mutant type *Pf*DHFR binding

Gly165 and Thr185. The Cys59Arg, Pro113 and Phe116 were also included into the systems for comparing their particular interaction enrgies contributed to Cyc. The 2D scheme of twenty-three residues was shown in Fig. 1.

III. Results and Discussion

The particular interaction energy which obtained from the Cyc ligand and each amino acids at MP2/6-31G(d,p) calculations, was define by

$$E^{INT}_{(ligand-\mathrm{amino}acid)} = E^{AB}_{(ligand-\mathrm{amino}acid)} - E^{AB}_{(ligand)} - E^{AB}_{(\mathrm{amino}acid)} \quad (1)$$

where A and B are the number of basis sets of ligands and amino acids, respectively, $E^{AB}_{(\mathrm{ligand-aminoacid})}$ is the energy of the ligand-amino acid complex and $E^{AB}_{(ligand)}$ and $E^{AB}_{(\mathrm{aminoacid})}$ are the energies of the ligand and the amino acid, respectively, with the basis set of A plus B.

The obtained results of particular interaction energy of Cyc and key amino acids surrounding the binding pocket of wild type and quadruple mutant *Pf*DHFRs were plotted in Fig. 2.

Asp54 showed the strongest interaction energy with the wild type and the mutant enzymes. In molecular level investigation, the Asp54 was formed a strong H-bonded interaction with the 2-amino group. Ile14 also formed H-bonded interaction with the 4-amino group. In the case of Phe58, it could be presented as a *pi-pi* interaction between the phenyl ring of Phe58 and the 1,3,5-dihydrotriazine ring of the inhibitor. Fig.3 showed the main interaction of Cyc and amino acids, Asp54, Ile14, Ile14Leu, and Phe58. Although Ile164 mutated to Leu164, its back bone amino acid still produced H-bonded interaction with the 4-amino group of the 1,3,5-dihydrotriazine ring. The two mutations at Asn51Ile and Cys59Arg did not have any significantly different interaction with Cyc in both the wild type and the mutant enzyme. The changing from Ser to Asn at the 108 position showed the largest difference in repulsive interaction energy of approximately 4 kcal/mol. This is due to a steric clash between the *p*-Cl phenyl substitute of Cyc and the larger side chain of Asn108

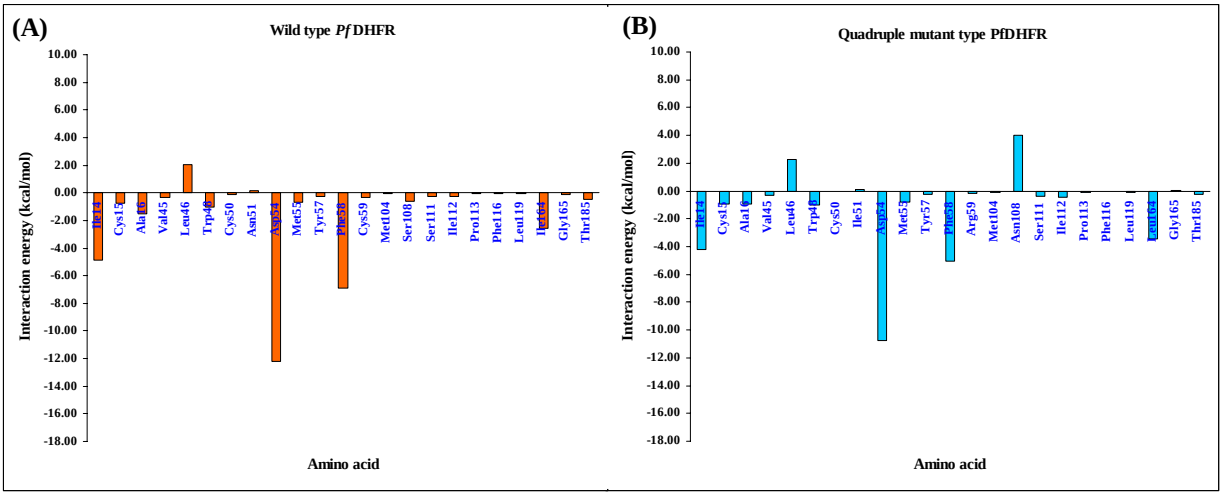

Fig. 2 The obtained MP2/6-31G(d,p) with BSSE-CP interaction energies of Cyc and individual amino acids surrounding the binding pocket of wild type (A) and quadruple mutant type (B) *Pf*DHFRs

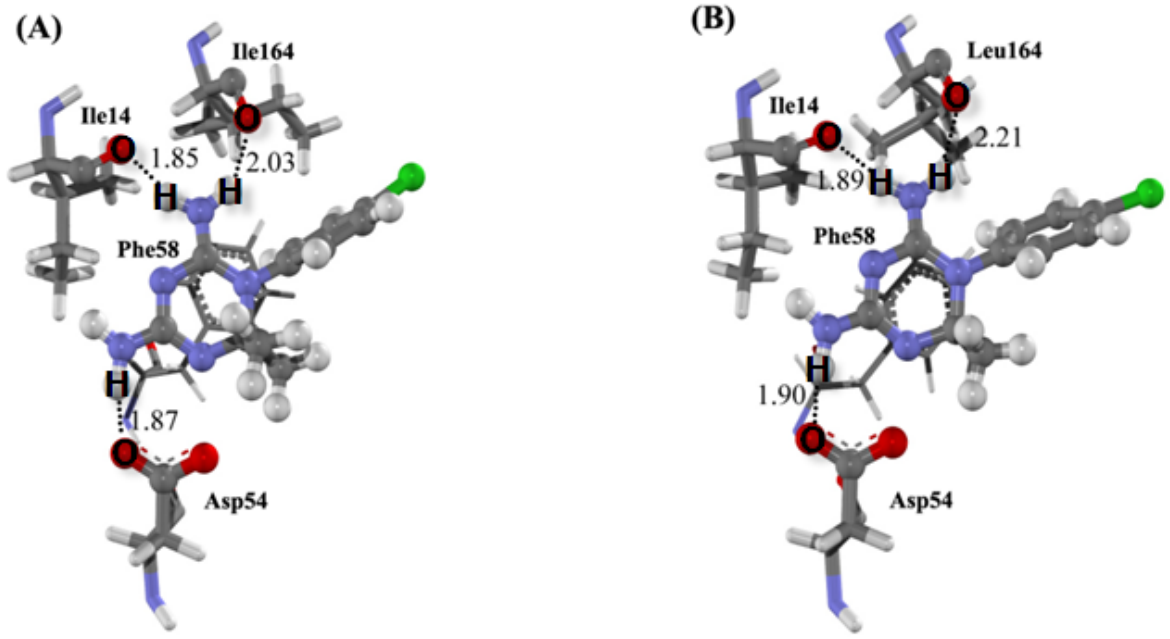

Fig. 3 H-bond distances between Cyc inhibitor and residues in the binding pocket; (A) wild type and (B) quadruple mutant type DHFRs (in Å).

IV. Conclusions

In this work, we can conclude that the particular interaction energy investigations using quantum chemical calculations can be used to explain the cause of the Cyc drug resistant in quadruple mutant PfDHFR which caused by the repulsive energy at the Ser108Asn and the *p*-Cl phenyl of Cyc. Therefore, this process of calculations can also be applied to other ligand-enzyme complexes for getting the insight molecular details.

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