CONCLUSION

Particular Interaction between Pyrimethamine Derivatives and Quadruple Mutant Type Dihydrofolate Reductase of *Plasmodium Falciparum*: CoMFA and Quantum Chemical Calculations Studies

The CoMFA analysis is a very powerful method for ligand-based drug design. Therefore, in this study, the CoMFA method was selected to build a linear equation of the quantitative structure activity relationship of the Pyr derivatives that are active against quadruple mutant type *Pf*DHFR. The combined C_{sp3} (+1), O_{sp3} (-1) and H (+1) probe atoms model was selected to represent the CoMFA moelcular fields for accounting the different types of interactions between mutant *Pf*DHFR binding site and Pyr derivatives. The steric contour maps of this model suggest that **X** and **R** substitutions favoured a bulky group which is opposite to **Y** substitution. Electrostatic maps are displayed surrounding the phenyl ring of the **R** substituent of the template compound which means that an electron donating substituent on the phenyl ring will increase the biological activity. Therefore, the characteristics of new design inhibitors are the bulky group on the **X** substitution, the hydrogen atom on the **Y** substitution and the long chain on the **R** substitution.

Moreover, we also performed MP2/6-31G(d,p) quantum chemical calculations with BSSE-CP energy correction to investigate the particular interaction energy of compounds **1** (Pyr) and **6** (Cl substituent on **X**). The obtained results clearly show that Asn108 is the caused of Pyr resistance with the highest repulsive interaction energy and negative electrostatic potential. Accordingly, this calculation is consistent with the unfavoured steric region of CoMFA contour maps. The CoMFA and particular interaction energy analyses will be useful for identifying the structural features of potent Pyr derivatives active against quadruple mutant type *Pf*DHFR which is an important target of malaria chemotherapy.

Interactions between Cycloguanil Derivatives and Wild-Type and Resistance-Associated Mutant *P. Falciparum* Dihydrofolate Reductases

An application of the CoMFA technique was performed on Cyc derivatives of both the wild type and the quadruple mutant type *Pf*DHFRs. Satisfactory CoMFA models of both the wild type and the mutant were obtained with LOO cross-validation r^2_{cv} values of 0.727 and 0.786, respectively. The steric and electrostatics contour maps of Cyc derivatives for wild type enzymes can be summarized that the Cl substitute at Y position is better than H, the long chain with O-linkage between the aromatic rings gives a good activity for R_2 and the bulky group is not suitable for R_1. On the other hand, the CoMFA contour maps of Cyc derivatives active against the quadruple mutant type *Pf*DHFR described the different Cyc structural requirements comparing with the wild type. For example, the use of a Cl substitute on *para*-phenyl or Y position decreased binding affinity against the quadruple mutant and the long aliphatic side chain is suitable for R_2, which differed from the result for the wild type activity. Therefore, CoMFA appears to be a valuable tool to help understanding of different structure-activity relationships.

In addition, the deep molecular energy investigations using quantum chemical calculations of the Cyc bound into to binding sites of wild type and the quadruple mutant *Pf*DHFRs are shown to be the resistant cause arose from the key mutant residue at Ser108Asn. This is caused by the steric clash between the *p*-Cl phenyl of Cyc and the side chain of the residue Asn108. On the other hand, WR99210, a potent flexible inhibitor does not result in a repulsive energy when the residue changes from Ser108 to Asn108.

The CoMFA and quantum chemical approaches are powerful methods for determining the ligand requirements of the structural steric and electrostatic properties in various types of enzymes and the details of the key molecular interactions which cannot be solved by experimentally.

The benefits of the two approaches are therefore complementary, and combined use of particular residue interacted with inhibitor and structural requirement of inhibitor which active against difference types of *Pf*DHFRs have provided valuable informations for guiding the new design compounds. Hence, the next step of our study will be focused on the validation of the design Cyc chemical structures based on the present models by docking analysis them into the *Pf*DHFR binding sites.

Understanding on Different Binding Energies of Cycloguanil, Pyrimethamine and WR99210 Antifolates to Quadruple Mutant Type *Pf*DHFR, Based on Three-Layer ONIOM Method

We investigated the different binding energy between the potent WR99210 inhibitor and the poor antifolates represent by Pyr and Cyc inhbitors, active against the quadruple mutant type of *Pf*DHFR. The AMBER molecular dynamics simulations is well bimolecular force fields for constructing the complex of three antifolates and mutant *Pf*DHFR with RMSDs of ligands and their protein using backbond amino acids plot less than 1 Å and 2 Å, respectively.

Consequently, the binding energy of all complexes was extrapolated using the ONIOM3 (B3LYP/6-31G(d,p):PM3:UFF) calculations. We found that the WR99210 gave highest total binding energy, high quantum chemical energy for small region which including Asp54, Asn108 and Leu164, and low quantum chemical energy for the intermediate region consisting the residues surrounding the binding pocket within at least one atom of ligand interact with any atoms of residues, comparing with Pyr and Cyc ligands. In addition, the loss of ligand/enzyme binding is interested to clear that any mutations lead to unstable of ligand in the binding pocket.

The obtained molecular dynamics minimizations and ONIOM3 binding energy extrapolations indicate that the mutated residue Asn108 is the main causes of less potency K_i for Pyr and Cyc drugs with loss of high quantum chemical energy for small region appearances. Especially, the obtained binding energies of three antifolates were accorded to the biological activity for the quadruple mutant *Pf*DHFR

as well. Therefore, the extrapolated energy using the ONIOM3 methods can be well investigated the different binding energy of the poor and potent inhbitors as using in term of the relative energy levels. However, the extrapolated binding energy of three antifolates which bound into the wild type *Pf*DHFR will be further investigated for more clearly resistance causes.