THESIS

MOLECULAR MODELLING AND QUANTUM CHEMICAL CALCULATIONS STUDY ON ANTIFOLATE ANTIMALARIAL INHIBITORS

PHORNPHIMON MAITARAD

GRADUATE SCHOOL, KASETSART UNIVERSITY

2008

THESIS

MOLECULAR MODELLING AND QUANTUM CHEMICAL CALCULATIONS STUDY ON ANTIFOLATE ANTIMALARIAL INHIBITORS

PHORNPHIMON MAITARAD

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Chemistry)
Graduate School, Kasetsart University
2008

Phornphimon Maitarad 2008: Molecular Modelling and Quantum Chemical Calculations Study on Antifolate Antimalarial Inhibitors. Doctor of Philosophy (Chemistry), Major Field: Physical Chemistry, Department of Chemistry. Thesis Advisor: Associate Professor Supa Hannongbua, Dr.rer.nat. 125 pages.

Comparative molecular field analysis (CoMFA) was performed on twenty-three pyrimethamine (Pyr) derivatives actived against quadruple mutant type (Asn51Ile, Cys59Arg, Ser108Asn, Ile164Leu) *plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR). The best model (r^2_{cv} = 0.702, S_{press} = 0.608, r^2_{nv} = 0.980, s = 0.156, and $r^2_{testset}$ = 0.698) with combined three types of probe atoms, C_{sp3} (+1), O_{sp3} (-1) and H (+1), can be use to explain steric and electrostatic structural requirements for Pyr compounds. In addition, the CoMFA mehtod was also performed on twenty-five cycloguanil (Cyc) derivatives derived both wild type (r^2_{cv} = 0.727 and r^2 = 0.985) and quadruple muatnt (r^2_{cv} = 0.786 and r^2 = 0.979) *Pf*DHFR models. These two models can be determined the different structural requirements for the potency of inhibiting between the wild type and the quadruple mutant *Pf*DHFRs very well.

Deeply in molecular details, an understanding of particular interaction energy between antifolate inhibitors and surrounding residues in the binding pocket was performed by using MP2/6-31G(d,p) accurate quantum chemical calculations. The obtained results clearly demonstrate that Asn108 is the caused of Pyr and Cyc resistances. Furthermore, we investigated the different binding energy between the potent WR99210 inhibitor and the poor inhibitors represented by Pyr and Cyc, active against the quadruple mutant type of *Pf*DHFR. The AMBER molecular dynamics simulations are well bimolecular force fields for constructing and optimizing the complexes of three antifolates and the mutant *Pf*DHFR. Consequently, the binding energy of all complexes was extrapolated using the ONIOM3 (B3LYP/6-31G(d,p):PM3:UFF) calculations. The WR99210/*Pf*DHFR gave highest total binding energy. Basically, the loss of ligand/enzyme binding is the key point 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 cause of the less potency K_i for Pyr and Cyc drugs which dued to the loss of high quantum chemical energy for small region (antifolate inhibitor + Asp54 + Asn108 + Leu164). Hence, this appearance accorded to experimental biological activity.

The goal of the research is the demonstrating the cause of the antifolate drugs resistance which came from the changing of Ser108 to Asn108 using the CoMFA and quantum chemical calculations studies. Moreover, the obtained CoMFA models can be used to guide the new designed potent antifolate inhibitors for the mutant enzyme.

_____ / / _____

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my supervisor, Associate Professor Dr. Supa Hannongbua. Her wide knowledge and her logical way of thinking have been of great value for me. Her understanding, encouragment and guidance have provided a good basis for the present research works. My supervisor not only guides me in aspect of scientific research but also teaches me many great things, such as, positive thinking, good performance, active coordination and kind attitude.

I am deeply grateful to my co-supervisor, Dr. Sumalee Kamchonwongpaisan, Senior Researcher at National Center for Genetic Engineering and Biotechnology (BIOTEC), for her detailed, valuable advice, kind help and constructive comments, and for her important collaboration throughout this work.

I owe my most sincere gratitude to Professor Dr. Yongyuth Yuthavong, who gave me a great an opportunity to work with his malaria research unit at National Center for Genetic Engineering and Biotechnology (BIOTEC) and his very kind help.

I warmly thank Dr. Chak Sangma for his program supporting around my work and interesting explorations in operations have been very helpful for this study.

My warm thanks are due to Professor Jumras Limtrakul, who provided facility on the supercluster computer calculations and gave me a great experience for PTT projects. I also wish to thank the Large Scale Laboratory, Natioanl Electronic and Computer Technology Center (NECTEC), for supporting Sybyl program throughout my research works.

I wish to thank Mr. Ben Parslew and Mr. Andy for revising the English of my manuscripts. I also thank to Dr. Thanyada Rungrotmongkol, Dr. Rungtiwa Chidtong, Dr. Pensri Srivub, Dr. Patchreenart Saparpakorn, Mr. Witcha Treesuwan, Mr. Bundet Boekfa, Ms. Pornthip Boonsri, Ms. Natcha Injan and Ms. Wanwimon Mokmak as

well as LCAC members whose names were not mentioned here for their friendships, valuable assistances and excellent helps.

During this work I recieved full supported from the Thai Graduate Institute of Science and Technology scholarship (TGIST), National Science and Technology Development Agency. I also have partial supported from the Commission on Higher Education (CHE), Graduate School of Kasetsart University and the Postgraduate Education and Research Program in Petroleum Petrochemical Technology and Advance Material.

I owe my loving thanks to my best friend Mr. Pakasit Jirask in manner of his kind assistance and his time spent in my thesis preparation behind the scenes without ever receiving and he always besides me when I fell down.

Finally, I would like to express my most profound gratitude to my lovely parents and two sisters for their love, sincere care, constant supports and great encouragement throughout the duration of my educations.

Phornphimon Maitarad

April, 2008

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

binding position of both two inhibitors.

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATION

2D	=	Two-dimension
3D-QSAR	=	Three-dimensional quantitative structure-activity relationship
Ala (A)	=	Alanine
Asn (N)	=	Asparagine
Arg (R)	=	Arginine
Asp (D)	=	Aspatic acid
B3LYP	=	Beck's three parameter hybrid functional using the LYP correlation functional
BSSE-CP	=	Basis set superposition error based on the counterpoise scheme
CoMFA	=	Comparative molecular field analysis
Comp	=	Compounds
C_{sp3} (+1)	=	Carbon sp3-hybridization with plus 1 charge probe atom
Cys (C)	=	Cysteine
Cyc	=	Cycloguanil
DNA	=	Deoxyribonucleic acid
DHF	=	Dihydrofolate
DHFR	=	Dihydrofolate reductase
dTMP	=	Deoxythymidylate
Gly (G)	=	Glycine
H (+1)	=	Hydrogen plus 1 charge probe atom
HF	=	Hartree-fock theory
HIV-1	=	Human immunodeficiency virus type 1
HQ	=	High level of quantum chemical calculations
Ile (I)	=	Isoleucine
K_i	=	Inhibition constant
Leu (L)	=	Leucine
LOO	=	Leave-one-out
LQ	=	Low level of quantum chemical calculations
Lys (K)	=	Lysine

LIST OF ABBREVIATION (Continued)

MD	=	Molecular dynamics
Met (M)	=	Methionine
MLR	=	Multiple linear regression
MM	=	Molecular mechanics
MP2	=	Second order Möller-plesset
NAD	=	Nicotinamide adenine dinucleophide
Noc	=	Number of component
ONIOM	=	Our own n-layer intergrated molecular orbital molecular mechanics
O_{sp3} (-1)	=	Oxygen sp3-hybridization with minus 1 charge probe atom
PDB	=	Protein data bank
Pf	=	*Plasmodium falciparum*
*Pf*DHFR	=	*Plasmodium falciparum* dihydrofolate reductase
Phe (F)	=	Phenylalanine
p*Ki*	=	Negative logarithm of inhibition constant
PLS	=	Partial least square
PM3	=	Modified neglect of diatomic overlap, parametric method number 3
PME	=	Particle mesh ewald
PRESS	=	Prediction error sum of squares
Pro (P)	=	Proline amino acid
Pyr	=	Pyrimethamine
QM	=	Quantum mechanics
QM/MM	=	Quantum mechanical/molecular mechanical method
QSAR	=	Quantitative structure-activity relationship
r^2_{cv}	=	Predictive ability of cross-validation
r^2_{nv}	=	Predictive ability of no-validation
RMS	=	Root mean square
RMSD	=	Root mean square deviation

LIST OF ABBREVIATION (Continued)

Ser (S)	=	Serine
SHMT	=	Serine hydroxymethyltransferase
S_{press}	=	Uncertainty of the prediction
SSY	=	Variance of the data around the mean value
THF	=	Tetrahydrofolate
Thr (T)	=	Threonine
Trp (W)	=	Tryptophan
TS	=	Thymidylate synthase
Tyr (Y)	=	Tyrosine
Val (V)	=	Valine
UFF	=	universal force field