

International Journal of Research in Pharmacy and Science

QSAR Studies of Potent Inhibitors of Malaria Parasite Dihydroorate Dehydrogenase By Comparative Analysis

Sharma Rajesh*, Patel Rajesh, Patil Swaraj

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshshila Campus,
Khandwa Road, Indore-452017, M.P., India

ABSTRACT

Three-dimensional quantitative structure–activity relationship (3D-QSAR) analyses were carried out on derivatives as Dihydroorate dehydrogenase inhibitors in order to elucidate their antimalarial activities. The most significant values of model 1 generated are internal predictivity 77.039% ($q_2 = 0.77039$) and external predictivity 89.00 % ($\text{pred}_r2 = 0.89$) variance in antimalarial activity. The comparative studies had performed with different software. By studying the QSAR model one can select the suitable parameter for designing active compound with maximum potency and relation of the physiochemical property with biological activity can be easily understood. It is hoped that the work presented here will play an important role in understanding the relationship of physiochemical parameters with structure and biological activity. By studying the QSAR model one can select the suitable parameter for designing active compound for antimalarial activity with maximum potency. By the help of the QSAR we can generate a most active compound which is helpful in the future designing of the new compound. The parameter which contribute maximum in the biological activity are molecular weight (MW), Principal Moment of Inertia – Z (PMIZ) and Non-1,4 Van der waal Energy N1,4 VDWE. Valstat software generates the biological equation and the correlation matrix which is beneficial in understanding the potency of the compound.

KEY WORD: Dihydroorate dehydrogenase inhibitors, antimalarial, external predictivity, potency, structure

*** Corresponding Author**

Dr. Rajesh Sharma,
School of Pharmacy, Devi Ahilya
Vishwavidyalaya, Takshshila Campus,
Khandwa Road, Indore, M.P., India-452 017
Tel. : +91 731 2100605
E-mail: rbsm73@yahoo.co.in

INTRODUCTION

Plasmodium falciparum DHODH activity can be destroyed by using inhibitor or lowering the expression level of *Plasmodium falciparum* Dihydroorotate dehydrogenase (DHODH), which inhibits parasite growth. So *Plasmodium* DHODH is a good target for drug development.¹⁻⁵ Pyrimidines are a key component of nucleic acids as well as phospholipids and glycoproteins. Humans can acquire pyrimidines via the salvage pathway as well as de novo biosynthesis of pyrimidines via the conserved pathway. However, *P. falciparum* lacks a salvage pathway for pyrimidines and is therefore totally reliant on the de novo biosynthetic pathway. Therefore, de novo pyrimidine biosynthesis represents an attractive and selective target for the development of new therapeutics against *P. falciparum*.⁶⁻⁸ The anti-malarial drug atovaquone occupies the quinol oxidase (Qo) site of mitochondrial cytochrome *b*, inhibiting electron flux through the cytochrome *bc*₁ complex (ubiquinol:cytochrome *c* oxidoreductase or complex III) and collapsing mitochondrial membrane potential with a potency 1000-fold greater in *Plasmodium* than mammalian cells.^{9,10} Unfortunately, high rates of recrudescence and treatment failure were seen after antimalarial use of atovaquone alone¹¹, and treatment failures after The de novo pyrimidine pathway appears to be important for several cellular functions. Pyrimidines are needed not only for RNA and DNA synthesis but also for protein glycosylation, membrane lipid biosynthesis, and strand break repair. Recent efforts for the development of antipyrimidine drugs with an anti-inflammatory, immunosuppressive, antiproliferative, or antiparasitic efficacy profile have focused on dihydroorotate dehydrogenase (DHODH), the fourth sequential step in the de novo pyrimidine nucleotide synthesis pathway, as a promising target enzyme to depress the intracellular level of pyrimidine nucleotides¹²⁻¹⁴. Dihydroorotate dehydrogenase catalyses the oxidation of dihydroorotate to orotate, the first aromatic intermediate in this biosynthetic pathway, with the aid of a flavin cofactor and an electron receptor.¹⁵ On the basis of sequence similarity, the DHODHs can be divided into two major families, i.e., family 1 (A and B) and family 2.¹⁶ This division correlates with subcellular location of the proteins as well as their preferences for electron acceptors. Enzymes of family 1, found in Gram-positive bacteria and in the anaerobic yeast *Saccharomyces cerevisiae*, are located in the cytosol of the cell. They have less than 20% sequence identity with the membrane-bound enzymes of family 2, found in eukaryotes and in some prokaryotes such as the Gram-negative bacteria related to *Escherichia coli*. The cytoplasmic enzymes utilize fumarate or NAD⁺ to oxidize FMN, whereas the membrane-bound enzymes, which are mitochondrial in eukaryotes, require quinones as their physiological oxidant¹⁷. Several additional classes of compounds have been found to inhibit this complex in *Plasmodium falciparum*, leading to

additional potential drugs in early stages of development. Inhibitors of another essential mitochondrial enzyme, dihydroorotate dehydrogenase (DHODH), are also advancing toward drug development.^{18,19}

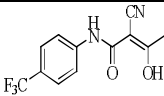
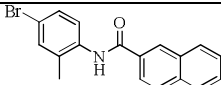
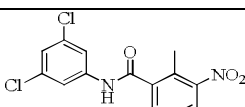
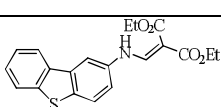
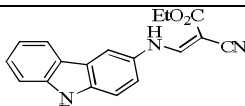
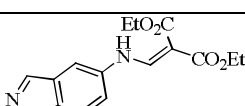
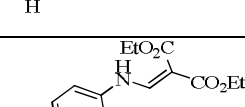
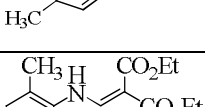
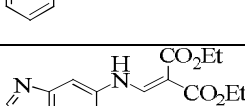
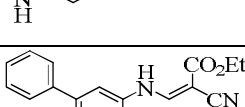
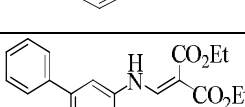
MATERIAL AND METHOD

The antimalarial activity data of derivatives as were taken from the reported work of Heikkila et al.²⁰ (Table 1). The biological activity data (IC_{50}) was converted to negative logarithmic mole dose (pIC_{50}) for quantitative structure activity relationship (QSAR) analysis. The molecular modeling study was performed using CS Chem Office version 8 while the regression analysis was carried out with VALSTAT and SPSS software.

The VALSTAT is interactive program, when initiated, asks for declaration in the format of (y/n). If user enters 'n', then program is terminated automatically. The main options that appear on the screen based on the user's interest are, (i) have you entered data in specific files (y/n), (ii) do you want test and training set (y/n), (iii) do you want intercorrelation matrix (y/n), (iv) are you going for simple Linear Regression Analysis (y/n), (v) are you going for Stepwise Linear Regression Analysis (y/n), (vi) enter correlation limit within the parameters (0-0.99), (viii) are you going for nonlinear Stepwise Regression Analysis(y/n), (ix) enter number of independent variables for Multiple Linear Regression Analysis (1-24), (x) do you want correlation matrix for Multiple Linear Regression parameter (y/n) and (xi) are you going for validation of model (y/n). Here it asks for training/test set (y/n). If answer is 'n', then program progresses to next step automatically. Now question appears on screen for correlation matrix (y/n), if reply is 'y' then program starts for finding of inter correlation within the independent parameters and the result of inter-correlation gets stored in the RESULT.TXT file in matrix form.

QSAR models were built using the regression analysis module of SPSS version 17. The correlation matrix was used to correlate the biological activity with the various predictor variables. The QSAR models were evaluated by using the statistical parameters viz., correlation coefficient (r) or coefficient of determination (r^2), adjusted r^2 (r^2 Adj), Standard error of estimate and Fischer F-value. The latter is used to assess the significance of the individual regression terms. The figures within the parentheses following the coefficient terms are the standard error of the regression terms and the constants. Durbin-Watson (DW) test was employed to check the serial correlation in residuals. Since the DW values in all our derived models are greater than 2.0, there is probably not any serious auto correlation in the residuals.

Table1: General structure of the compounds of derivatives and their biological activities (data set of 11 molecules)

S.NO	Structure	IC ₅₀ (μm)	pIC ₅₀
1.		190.1	3.721018
2.		0.18	6.638272
3.		0.23	3.721018
4.		0.16	6.79588
5.		0.44	6.356547
6.		27.78	4.556268
7.		40	4.39794
8.		345.6	3.461426
9.		349.1	3.45705
10.		21.3	4.67162
11.		86.7	4.061981

RESULT AND DISCUSSION

The result of valstat model is as:

Model 1 is a monoparametric equation developed for available antimalarial activity data. The most significant values of model generated are internal predictivity 77.039% ($q^2 = 0.77039$) and external predictivity 89.00 % ($pred_r^2 = 0.89$) variance in antimalarial activity.

Model: 1 BA = [1.94151(± 1.45976)] +PMIZ [0.000620399(± 0.000248639)] +N1,4VDWE [-0.52281(± 0.247224)]

Contribution of parameters to model is PMIZ:N1,4VDWE::5.52713:1

n=11, r=0.946203, $r^2=0.895301$, variance=0.214732, std=0.463392, F=34.2047, FIT=456.063, $q^2 = 0.770396$, Spres=0.686226, SDEP=0.585216

Table 2: correlation matrix of model 1

	PMIZ	N1,4VDWE
PMIZ	1.000000	
N1,4VDWE	0.126656	1.000000

Model 2 is a monoparametric equation developed for available antimalarial activity data. The most significant values of model generated are internal predictivity 74.0% ($q^2 = 0.74$) and external predictivity 87.31 % ($pred_r^2 = 0.8731$) variance in antimalarial activity.

Model: 2 BA = [2.62926(± 1.34973)] +PMIY [0.000579024(± 0.000261937)] +N1,4VDWE [-0.502971(± 0.273597)]

contribution of parameters to model is PMIY:N1,4VDWE::4.53782:1

n=11, r=0.934399, $r^2=0.873101$, variance=0.260263, std=0.51016, F=27.5211, FIT=366.948, $q^2=0.740636$, Spres=0.729343, SDEP=0.621986

Table 3: Correlation matrix of model 2

	PMIY	N1,4VDWE
PMIY	1.000000	
N1,4VDWE	0.161986	1.000000

Model 3 is a monoparametric equation developed for available antimalarial activity data. The most significant values of model generated are internal predictivity 67.01% ($q^2 = 0.6701$) and external predictivity 84.01 % ($\text{pred_}r^2 = 0.8401$) variance in antimalarial activity.

Model: 3 BA = [-2.51614(± 4.31312)] +MW [0.0253119(± 0.0136511)] +N1,4VDWE [-0.569884(± 0.30799)]

contribution of parameters to model is MW:N1,4VDWE::11.834:1

n=11, $r=0.913973$, $r^2=0.835346$, variance=0.337697, std=0.581117, F=20.2934, FIT=270.578, $q^2=0.671932$, Spres=0.820275, SDEP=0.699533

Table 4: Correlation matrix of model 3

	MW	N1,4VDWE
MW	1.000000	
N1,4VDWE	0.054380	1.000000

The result of SPSS model is as:

Model 1 The most significant values of model generated external predictivity 84.01 % ($\text{pred_}r^2 = 0.873$) variance in antimalarial activity.

Model 1 $2.629 \pm 0.571 + \text{PMIY}[0.001(\pm 0.000)] + \text{NVWDE}[(-0.503) \pm (0.116)]$

n=11, $r=0.934$, $r^2=0.873$, Adj $r^2 = 0.841$, F=27.521, Durbin Watson constant= 2.040, Standard error of estimate=0.5101

Finally, it is hoped that the work presented here will play an important role in understanding the relationship of physiochemical parameters with structure and biological activity. By studying the QSAR model one can select the suitable parameter for designing active compound for antimalarial activity with maximum potency.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, School of Pharmacy for providing facilities and MPCST. Swaraj Patil is grateful to the University Grants Commission, New Delhi for fellowship.

REFERENCE

1. Krungkrai, J. Purification, characterization and localization of mitochondrial dihydroorotate dehydrogenase in *Plasmodium falciparum*, human malaria parasite. *Biochim Biophys Acta*. 1995; 1243: 351-60.
2. McRobert, L, McConkey GA. RNA interference (RNAi) inhibits growth of *Plasmodium falciparum*. *Mol. Biochem. Parasitol*. 2002; 119: 273-8.
3. Baldwin J, Farajallah AM, Malmquist NA, Rathod PK, Phillips MA. Malarial dihydroorotate dehydrogenase. Substrate and inhibitor specificity. *J. Biol. Chem*. 2000; 277: 41827-34.
4. Norager S, Jensen KF, Bjornberg O, Larsen SE. coli dihydroorotate dehydrogenase reveals structural and functional distinctions between different classes of dihydroorotate dehydrogenases. *Structure*. 2002; 10: 1211–1223.
5. Mather MW, Darrouzet E, Valkova-Valchanova M, Cooley JW, McIntosh MT, Daldal F. Uncovering the molecular mode of action of the antimalarial drug atovaquone using a bacterial system. *J Biol Chem*. 2005; 280: 27458–65.
6. Mather MW, Henry KW, Vaidya AB. Mitochondrial drug targets in apicomplexan parasites. *Curr Drug Targets*. 2007; 8: 49–60.
7. Van Dooren GG, Stimmler LM, McFadden GI. Metabolic maps and functions of the *Plasmodium* mitochondrion. *FEMS Microbiol Rev*. 2006; 30: 596–630.
8. Fry M, Pudney M. Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone. *Biochem Pharmacol*. 1992; 43: 1545–53.
9. Srivastava IK, Rottenberg H, Vaidya AB. Atovaquone a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *J Biol Chem*. 1997; 272: 3961–6.
10. Looareesuwan S, Viravan C, Webster HK, Kyle DE, Hutchinson DB, Canfield CJ. Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. *Am J Trop Med Hyg*. 1996; 54: 62–6.
11. Fivelman QL, Butcher GA, Adagu IS, Warhurst DC, Pasvol G. Malarone treatment failure and in vitro confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. *Malar J*. 2002; 1:1-10.

12. J.P. Davis GA, Cain WJ, Pitts RL, Magolda RA. Copeland, The Immunosuppressive metabolite of Leflunomide is a potent inhibitor of human dihydroorotate dehydrogenase, *Biochemistry*. 1996; 35: 1270–1273.
13. Olliaro P, Wirth D. New targets for antimalarial drug discovery. *J. Pharm. Pharmacol.* 1997; 49: 29–33.
14. Copel RS, Black CG. Parasites genomes. *Int. J. Parasitol.* 2005; 35: 447–465.
15. Bjoörnberg O, Rowland P, Larsen S, Jensen KF. Active site of dihydroorotate dehydrogenase A from *Lactococcus lactis* investigated by chemical modification and mutagenesis, *Biochemistry*. 1997; 36: 2899–2908.
16. Nagy M, Lacroute v, Thomas D. Divergent evolution of pyrimidine biosynthesis between anaerobic and aerobic yeasts. *Proc. Natl. Acad. Sci. USA.* 1992; 89: 8966–8970
17. Vaidya AB, Mather MW. Mitochondrial evolution and functions in malaria parasites. *Annu Rev Microbiol.* 2009; 63: 249–67.
18. Gujjar R, Marwaha A, El Mazouni F, White J, White KL, Creason S. Identification of a metabolically stable triazolopyrimidine-based dihydroorotate dehydrogenase inhibitor with antimalarial activity in mice. *J Med Chem.* 2009; 52: 1864–72.
19. Deng X, Gujjar R, El Mazouni F, Kaminsky W, Malmquist NA, Goldsmith EJ. Structural plasticity of malaria dihydroorotate dehydrogenase allows selective binding of diverse chemical scaffolds. *J Biol Chem.* 2009; 284: 26999–7009.
20. Heikkila T, Ramsey C, Davies M, Galtier C, Andrew M, Stead W, Peter JA, Colin WGF, Andrew NB, and Glenn AMC. Design and Synthesis of Potent Inhibitors of the Malaria Parasite Dihydroorotate Dehydrogenase. *J. Med. Chem.* 2007; 50: 186-191.