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## Note

# 3D-QSAR analysis of cycloguanil derivatives, highly active agents against A16V + S108T mutant of dihydrofolate reductase resistant strain (T9/94) of *Plasmodium falciparum*

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3D-Quantitative-structure activity relationship of some 4,6-diamino-1,2-dihydrotriazine derivatives (cycloguanils) having good activity against resistant strain of *P. falciparum* has been performed. The model developed has shown that the descriptors, ovality (steric descriptor), dipole-dipole energy (thermodynamic descriptor) contributing positively while stretch bend energy (thermodynamic descriptor) negatively to the biological activity. Statistical analysis has shown the model to be fit (R=0.924, $R^2$=0.853, F-test=18.840, t-test=4.340, stdev = 0.244, variance=0.051) and predictable ($R^2_{LOO}$=0.693, $R^2_{pred}$=0.265). It can be concluded that parent nuclei having functional groups with optimum values for these descriptors, might have better biological activity against resistant strains.

**Keywords**: 3D-QSAR, *Plasmodium falciparum*, dihydrofolate reductase, A16V + S108T mutant, multiple regression analysis

Malaria remains the world's most devastating human infection affecting over 200 million people worldwide and causing more than 2 million mortalities each year, especially in developing countries. Not only this, burden on the current therapy for malaria caused emergence of resistant strain of *P. falciparum*. Also the spreading of malaria to new geographical locations favored the chances of resistance development[1]. Extensive work been done to elucidate the *de-novo* and alternate mechanisms of biochemical reactions in the malarial protozoan, but the studies failed to develop effective "targets" for new therapy. Thus, these facts are forcing the development of new chemical agents effective against resistant strains.

Pyrimethamine (pyr), cycloguanil (Cyc) and other antifolates received considerable attention, as cost effective antimalarial agents used for prophylaxis and treatment of *P. falciparum* infection. These agents selectively inhibit plasmodial dihydrofolate reductase (DHFR), but the rapid emergence of antifolate resistant *P. falciparum* has unfortunately compromised the clinical utility of these drugs and thus highlights the urgent need to develop the anti-folate antimalarials to be effective against resistant strains. Evidence available suggested that parasites with A16V + S108T double mutation in the *dhfr* genes are resistant to Cyc[2-11]. Yuthavong recently pointed out that A16V mutation leads to steric interaction between Val-16 and one of the C-2 methyl groups of cycloguanil (steric constraint hypothesis). Moreover, S108T mutation is considered to decrease cycloguanil binding further through the effect on the orientation of the *p*-chlorophenyl group. Based on these findings, they synthesized some 4,6-diamino-1,2-dihydrotriazines with improved activity against resistant strains, by moving the *p*-chloro-substituent to the *m*-position in the chlorophenyl group, the orientation effect is reinforced by the *p*-chloro substituent in the 3,4-dichlorophenyl groups. The lead 1-(3,4-dichlorophenyl)-6, 6-dimethyl-1,6-dihydro-1, 3, 5-triazine-2,4-diamine, generated showed inhibitory activity similar to that of cycloguanil against the wild-type DHFR and about 120-fold more effective than cycloguanil against the A16V+S108T mutant enzyme and is about 85-fold greater than cycloguanil in *P. falciparum* clone (T9/94 RC17), which harbors the A16V+S108T DHFR[12,13].

This paper describes 3D-QSAR analysis performed using the earlier reported compounds (**Tables I** and **II**), which were synthesized and biologically evaluated against A16V + S108T mutant enzymes[14]. The mathematical model was assured for its predictability. The descriptors selected by the present model were ovality, stretch bend energy and dipole-dipole energy. The model was used to design new compounds, which are predicted to have better activity.

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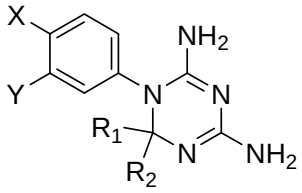
**Abbreviations:**

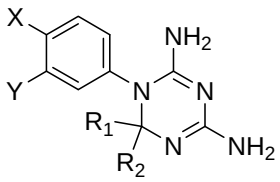
DHFR - Dihydrofolate reductase; $E_d$-Dipole-dipole energy; $E_s$- Stretch bend energy; Cyc- Cycloguanil; Pyr- Pyrimethamine; LOO- Leave one out; PRESS- Predicted sum of square stdev- Standard deviation; $r^2$- Correlation coefficient;. RMS- Root mean square.

**Table I —** Training set for 3D-QSAR analysis (growth inhibition values, $IC_{50}$, forA16V + S108T mutant enzyme-*)




| Compd | X | Y | $R_1$ | $R_2$ | $IC_{50}$ (nM) | Ovality | Stretch bend energy (kcal/mol) | Dipole-dipole energy (kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| **1** | Cl | H | H | H | 313 | 1.374 | 9.422 | -6.784 |
| **2** | Cl | H | H | Me | 347 | 1.357 | 9.171 | -6.737 |
| **3** | Cl | H | H | Et | 486 | 1.385 | 9.531 | -6.746 |
| **4** | Cl | H | H | $Pr^n$ | 365 | 1.420 | 9.843 | -6.817 |
| **5** | Cl | H | H | $Bu^t$ | 250 | 1.448 | 9.666 | -6.746 |
| **6** | Cl | H | H | $Pr^i$ | 2818 | 1.348 | 10.204 | -6.805 |
| **7** | Br | H | H | Me | 277 | 1.363 | 9.144 | -6.761 |
| **8** | Br | H | H | Et | 220 | 1.391 | 9.431 | -6.765 |
| **9** | Br | H | H | $Pr^n$ | 250 | 1.426 | 9.744 | -6.838 |
| **10** | Br | H | H | $Pr^i$ | 725 | 1.356 | 10.103 | -6.825 |
| **11** | Br | H | H | Ph | 185 | 1.453 | 9.387 | -6.982 |
| **12** | Me | H | H | Me | 469 | 1.385 | 9.135 | -7.127 |
| **13** | Me | H | H | Et | 517 | 1.399 | 9.528 | -7.115 |
| **14** | Me | H | H | Prn | 152 | 1.432 | 9.820 | -7.175 |
| **15** | Me | H | H | Pri | 3446 | 1.362 | 10.187 | -7.149 |
| **16** | F | H | Me | Me | 1001 | 1.329 | 9.932 | -6.796 |
| **17** | H | H | H | H | 356 | 1.347 | 9.420 | -7.061 |
| **18** | Cl | Cl | H | Me | 19 | 1.373 | 9.489 | -3.912 |
| **19** | H | Cl | H | Ph | 24 | 1.448 | 9.413 | -6.782 |
| **20** | Cl | Cl | H | Ph | 29 | 1.464 | 9.652 | -3.934 |

**Table II —** Test set for 3D-QSAR analyses




| Compd | X | Y | $R_1$ | $R_2$ | $IC_{50}$ (nM) | Ovality | Stretch bend energy (kcal/mol) | Dipole-dipole energy (kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| **21** | Cl | H | Me | Me | 2430 | 1.343 | 9.529 | -6.791 |
| **22** | Cl | H | H | $Bu^t$ | 65386 | 1.446 | 9.515 | -6.968 |
| **23** | Cl | H | H | Ph | 44 | 1.351 | 9.306 | -6.832 |
| **24** | Br | H | Me | Me | 2759 | 1.357 | 9.629 | -7.103 |
| **25** | Me | H | Me | Me | 3617 | 1.457 | 9.487 | -7.321 |
| **26** | Me | H | H | Ph | 39 | 1.318 | 9.453 | -7.071 |
| **27** | H | H | Me | Me | 445 | 1.355 | 10.106 | -6.742 |
| **28** | F | H | H | H | 312 | 1.343 | 10.681 | -6.768 |
| **29** | H | Cl | Me | Me | 298 | 1.342 | 9.643 | -6.667 |
| **30** | Cl | Cl | Me | Me | 307 | 1.360 | 9.921 | -3.788 |
| **31** | H | Cl | H | Me | 28 | 1.347 | 9.960 | -9.597 |

## Results and Discussion

A number of discrete equations have been derived using stepwise multiple regression analysis and specified criteria. Amongst all these equations, Model I (Eqn 1) has been screened as the most suitable for defining the biological activity. According to this relationship, among all the descriptors, the descriptors ovality, stretch bend energy and dipole-dipole energy are contributors to the activity. The model was found to fit values 0.799, 21.192, 3.729 and 0.285, for parameters $r^2$, F-test, t-test and stdev, respectively. On statistical evaluation of the model, compound **19** was found to be an outlier (Z-score = 2.600, acceptance criteria of ± 2.5 or below). So the compound **19** was removed from the series and the series of 19 compounds in training set was again analyzed using multiple regression analysis.

$$pIC_{50} = 6.190\ (\pm\ 3.292)\text{Ovality} + 0.343\ (\pm\ 0.144)E_d - 0.700\ (\pm\ 0.406)E_s - 2.094\ (\pm\ 6.568) \quad \ldots (1)$$

n =20, r = 0.894, $r^2$ = 0.799, F-test = 21.192, t-test = 3.729, stdev = 0.285,variance = 0.081

After eliminating compound **19**, the regression resulted in more predictive and informative equation as Model II **(**Eqn 2), having the same descriptors as Model I. The fitting $r^2$, now improved to 0.853 ($r^2$ = 0.799, Model I). The values for F-test, t-test and stdev, were also in agreement with the fitness of model. The predictability of Model II was challenged using both the "leave one out" and "external test set" validation procedures. The acceptable values of $r^2_{LOO}$ = 0.675 (**Figure 1**) and $r^2_{pred}$ = 0.258 (**Figure 2**), showed a confidence in the model.

A high value of $r^2_{bs}$ = 0.883 (**Table III**) further assured good predictability of the model.

$$pIC_{50} = 4.754\ (\pm\ 2.767)\text{Ovality} + 0.366\ (\pm\ 0.116)E_d - 0.642\ (\pm\ 0.0.325)E_s - 0.541\ (\pm 5.313) \quad \ldots (2)$$

n =19, r = 0.924, $r^2$ = 0.853, F-test = 18.840, t-test = 4.340, stdev = 0.244, variance = 0.051

Ovality, the ratio of the molecular surface area to the minimum surface area (surface area of a sphere having a volume equal to the solvent excluded volume of the molecule) which is a steric parameter, describes more the shape of molecule rather than the bulk of the molecule and can be correlated to the orientation of functional groups. The contribution of ovality to the activity of the molecule, therefore, suggests that to improve the activity of the molecule there is a requirement for proper orientation of the functional groups. This could be fundamentalised by the fact that presence of chloro group in the *meta* position of the phenyl ring as in compound **31** favored

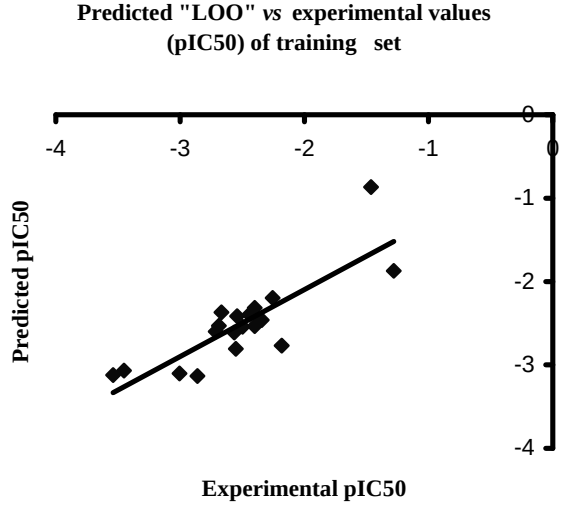



**Figure 1 —** Predicted "LOO" *vs* experimental values ($pIC_{50}$) of training set

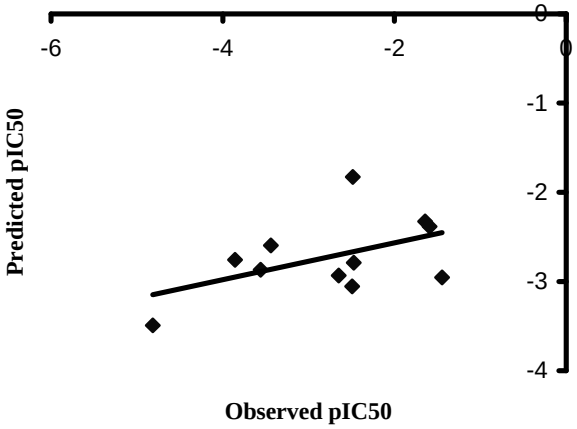



**Figure 2 —** Predicted *vs* experimental values ($pIC_{50}$) of test set

**Table III —** Internal and external validation statistics of the Model II

| | |
|---|---|
| $r^2_{LOO}$ | 0.675 |
| $S_{PRESS\ LOO}$ | 0.337 |
| $S_{DEP\ LOO}$ | 0.300 |
| $r^2_{PRED}$ | 0.258 |
| $r^2_{bs}$ | 0.883 |
| chance | 0.01 |

higher activity, as compared to compound **2** having the chloro group in the *para* position. This is probably because the change creates proper orientation of chloro group as seen by the improved value of ovality = 1.347 for compound **31** in comparison to 1.357 for compound **2**. Since ovality contributed maximum to the activity (82.65%) so even this small change is significant enough to improve the activity drastically. Also, favored values for dipole-dipole energy and stretch bend energy along with improved value of ovality for compound **31** improves its activity 13 times in comparison to compound **2**. Similar behavior is displayed by compound **29** and compound **21**. However, the theory encounters an exception when compound **19** and compound **23** are compared, since compound **19** is an outlier.

The significance of dipole-dipole energy, $E_d$, which can be defined as the sum of the electrostatic energy terms resulting from interaction of two dipoles, in drug enzyme interaction could be theorized by the fact that presence of *p*-chloro group in addition to *m*-chloro in compound **18** creates a greater dipole in the molecule and thus increases the activity, though to a very small extent, as compared to compound **31**. Since compound **19** is outlier, the exception to this relation can be observed for compounds **19** and **20**.

The effect of the stretch bend energy, $E_s$, (sum of the stretch bend coupling terms of the force field equations), on the activity can be explained by comparing the activity of compounds **1** to **6**, having the difference only in substitution at $R_2$ position by homologous alkyl groups. On moving higher up the homologous series the activity decreases with a few exceptions (compounds **4** and **5**).

From the comparisons made, it can be concluded that the three parameters (ovality, dipole-dipole energy and stretch bend energy) are contributing to the activity but the activity of the molecule can be increased only if the functional groups have optimum value for these descriptors. Based on these observations, it was possible to design two compounds as shown in **Table IV**, having activity thousands of times higher than cycloguanil.

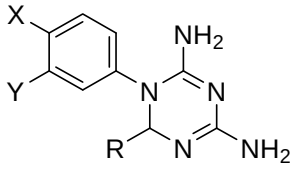
## Methodology

**Computer aided molecular modeling**. All the studies related to molecular modeling had been done using Chemoffice version 6.0 developed by Cambridge Corporation, USA. The molecular data set was first constructed in 2-D using ChemDraw version 6.0. These structures were converted to 3D using Chem3D version 6.0. The geometry optimization of these structures was done using semi-empirical approach based on Austin Model 1 approach by considering Mulliken charges, using CS MOPAC version 6.0. The convergence criterion was set as RMS gradient to be 0.001. The geometry optimized structures were subjected to single point calculation for descriptors calculation based on different servers *viz.* Chem. Prop Std, Chem. Prop pro, MM2, MOPAC, Gamess.

## Statistical Analysis

To derive a simple, unique and robust model with good predictability, stepwise regression based multiple regression analysis was used. The compounds were divided randomly in two data set groups, the one containing 20 compounds as training set (**Table I**) and the other containing 11 compounds as external predictive test set (**Table II**). Both the sets were analyzed for compounds with a good variation in their biological activity. The external predictive test set had almost 35% compounds in the data set. The compounds of the training set were used to predict model while those of the test set were used to cross-validate its predictability.

**Table IV —** Predictive activity of the designed compounds




| Compd | X | Y | R | $IC_{50}$ (nM) | Ovality | Stretch bend energy (kcal/mol) | Dipole-dipole energy (kcal/mol) |
|---|---|---|---|---|---|---|---|
| **D-1** | Cl | Cl | Cl | 0.17 | 1.4122 | 6.9643 | -2.5461 |
| **D-2** | Cl | Cl | Et | 0.25 | 1.3847 | 7.1532 | -2.3152 |

The growth inhibition values, $IC_{50}$, for A16V + S108T mutant enzyme of the compounds (obtained from literature) were used as the dependent variables to develop QSAR model for cycloguanil derivatives, as antifolate antimalarials, active against resistant strains of *P. falciparum.*

## Conclusion

Through the iterative computational approach, it was possible to extract a simple and highly informative model, having a high degree of predictability for the activity of cycloguanil derivatives against resistant strains of *P. falciparum*. The correlation developed was concurrent to the earlier findings, describing the effect of steric and orientation factors on the activity, but the novelty of its quantitative nature could be utilized more rationally to develop more active compounds. The descriptors selected by the model were ovality, stretch bend energy and dipole-dipole energy. These descriptors can be correlated with the bulk as well as orientation of the functional groups in the parent nuclei. Thus, it can be concluded that introduction of suitable functional groups having optimum activity for all these descriptors can be used to increase the activity of cycloguanil derivatives. This could be assured by the designed compounds predicted to be thousands of times more active than cycloguanil against resistant strains of *P. falciparum.*

## Acknowledgement

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