CHAPTER V

DISCUSSION AND CONCLUSION

5.1 Chloroquine Analog Antimalarial Drugs

5.1.1 Electron Distribution in the Quinoline Ring

The comparison of electron densities and the net charges of each atom in the chloroquine drug molecules (Table 4.1 and Figure 5.1) either by CNDO/2 or ab initio methods has shown that atoms in the heterocyclic ring (N1, C2, C3, C4, C7, C9, C10) and N2 have significant charge more than any other atoms in the quinoline ring. These atoms could be related to antimalarial activity. This can be seen from the various models tested by the inclusion of all carbon atoms in the aromatic ring. The compounds' dipole moment could also have a significant influence was therefore included in our linear model.

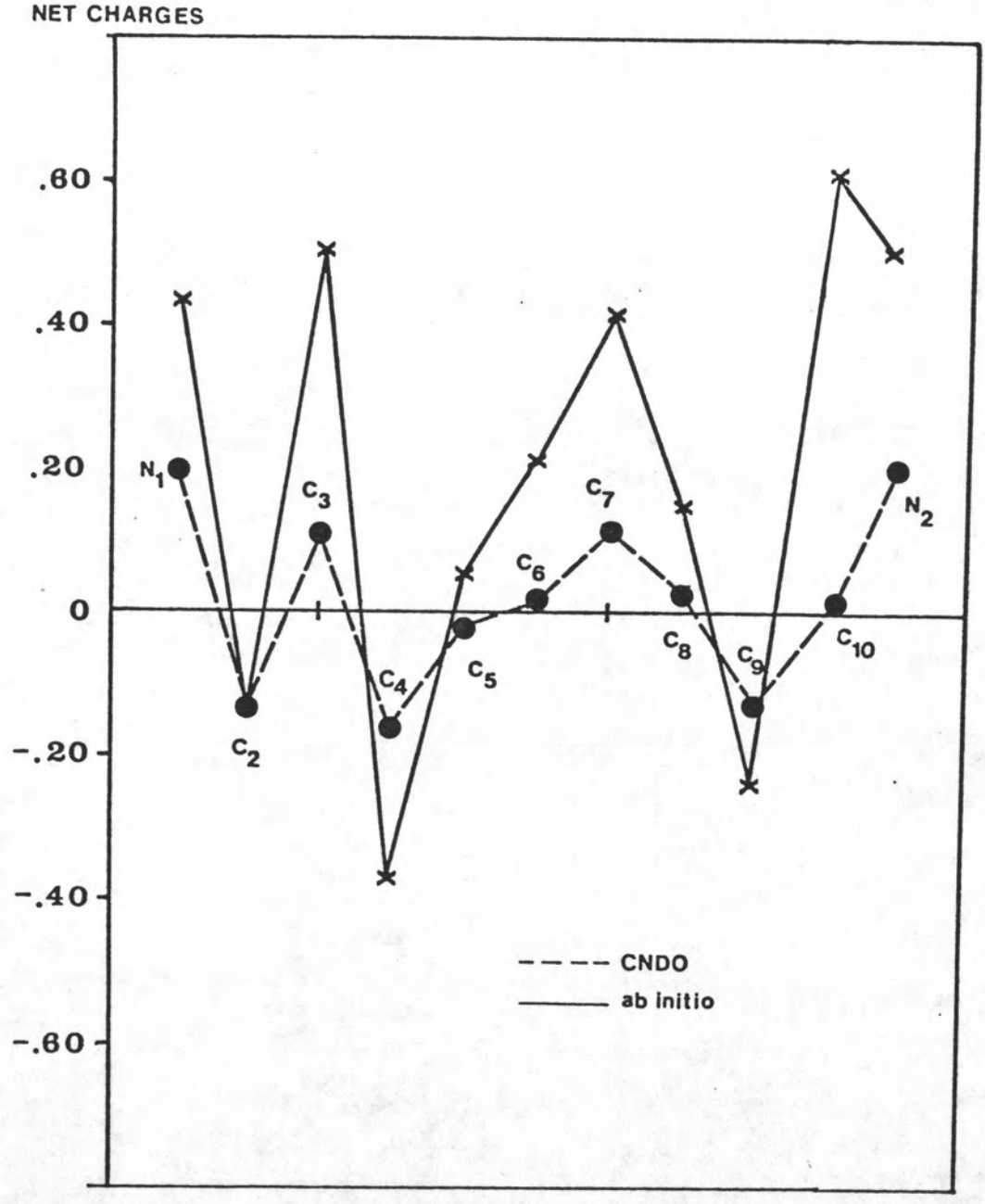


Figure 5.1 Net charges at various atoms in quinoline ring, and amino nitrogen, N2 of chloroquine

5.1.2 Electron Distribution - Activity Relationships of Chloroquine Antimalarial Drugs According to CNDO/2 Calculation

Fitting of all 22 chloroquine compounds by linear regression analysis at various steps led to the parameters and to an average deviation of calculated from observed drug activities, Cav given in Tables 4.2a - 4.2d, supplemented by the value of maximal deviation observed, Cmax. The relative importance of atoms in the molecule is recognized by their parameter size.

Model 0 which included all carbon atomic charges of quinoline ring plus N2 gave the parameter size of

$$C4 > N1 > C9 > C10 > C2 > N2,C3 >> C5,C6,C7,C8$$

Further inclusion of u does not signigicantly improve the fitting.

As mention before, a good model should normally contain as few parameters and variables as possible especially in our case when there are only a shell number of pharmacological data. In order to decrease the number of parameters, some atoms which have the parameter size close to each other, have been grouped together in the fitting models, i.e. qC(2,3), qC(5,6,7,8) (referred as qrest), and qC(9,10)

Comparing the various models with regard to the weight of the atomic parameters (Tables 4.2b-4.2d), the dominant parameters of the carbon atoms C9, C4 and C10 over C2 and C3 is of interest as well as the importance of nitrogen N2, relative to N1. According to the demand of the standard deviation of ln A less than 1, it seems inevitable to consider ring atom charges of the heterocyclic ring

plus nitrogen 2 for obtaining a satisfactory model. This has been realized in model 12;

$$\ln A = 40.831*qN1 + 46.279*qC2 + 10.239*qC3 + 184.991*qC4 - 162.808*qC9 - 110.202*qC10 + 18.348*qN2 + 7.775 \quad (5.1)$$

SD = 0.874 Cmax = 1.271 N = number of compounds = 22

This result may also indicate which part of the chloroquine molecules has to be considered as having the most influence on the activity or active center and may, therefore, be a help in the search for the yet unknown receptor of these drugs in the plasmodia. The data supplies not only geometry but also polarity of the active drug center.

Charges of qC(5-8) or qrest and dipole moment of the compounds seem to be of less importance for the pharmacological activity. Their inclusion into our linear model, either in the simpler ones (model 3, 5, 8, 10) or as addition in model 12 (model 13-15), do not significantly reduce the values of Cav and Cmax.

As a result of this evaluation we suggest that model 12 can serve as a useful basis for further evaluation.

The plot of calculated versus observed antimalarial activity (ln A) for model 12 is presented in Figure 5.2a.

Figure 5.2b shows the plot of $\ln A_{obs} - \ln A_{calc}$ or $\Delta \ln A$ versus $\ln A_{cal}$ for model 12.

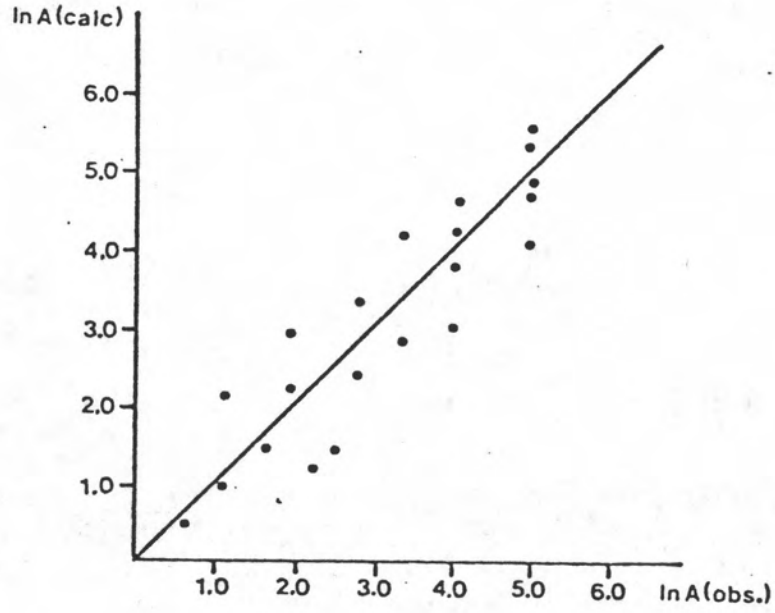


Figure 5.2a The plot of calculated versus observed antimalarial activity (ln A) for model 12

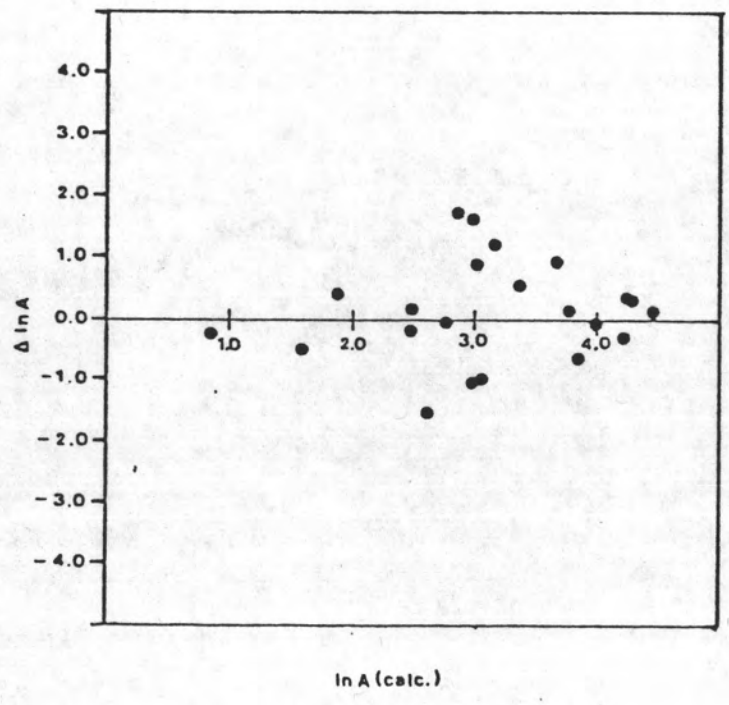


Figure 5.2b The plot of $\Delta \ln A$ versus $\ln A_{cal}$ for model 12

5.1.3 Comparison of Electron Distribution - Activity of Chloroquine Antimalarial Drugs According to CNDO/2 and Ab Initio Calculations

From our work with the chloroquines using CNDO/2 calculations, we have obtain some relationship between electronic distribution and antimalarial activity, indicating the active site of the drug. We are now in position to confirm our results for the ab initio - SCF-LCAO-MO calculations as we know that this calculation gives more correct results than any other approximation methods.

Given the limitation of CPU time for calculation of these the large molecules, only 17 chloroquine drugs have been calculated by ab initio method. Therefore the consecutive steps of fitting have been carried out other for other 17 chloroquines by and ab initio method by the same procedure as the 22 chloroquines.

Comparing the 22 and 17 chloroquine compounds calculated by CNDO/2 method, the fitting results (Tables 4.4a - 4.4c) led to the similar conclusion that the main influence parameters of equations are still characterized by C4, C9, C10, N1, N2 whereas C2 and C3 have additional but minor influence. Inclusion of C5-C8 and u is more efficient in this case. Still-due to the small number of pharmacological data, 17 chloroquine compounds, and in order to obtain equal model conditions as the 22 chloroquine data, model 12' is therefore favoured.

Model 12' can be expressed as followed:

$$\ln A = 188.354*qN1 + 155.211*qC2 + 73.128*qC3 + 2252.364*qC4 - 162.364*qC9 - 178.153*qC10 - 291.076*qN2 + 68.022 \quad (5.2)$$

SD = 0.886 Cmax = 1.750 N = 17

As a result of this evaluation, the 17 chloroquines fitting equation is now agreed with that of 22 compounds results so it could serve as the representation of fitting by CNDO/2 calculation to compare with those by ab initio.

Consider the linear models of ab initio charges density in tables 4.5a - 4.5c, the fitting models describes the parameter size of C4, C9, C10 and N2 to be greater than those of C2, C3 and C(5-8). In table 4.5c, the model 12" is of interest because it cansists of the same parameter types as model 12 and 12' and the Cav value is in the satisfactory range.

According to the linear fitting equations, the data for Cav and Cmax are not improved by the ab initio approach campared to CNDO/2 data and the relative important of parameters is also comparable. The enormous increase of computational effort seems therefore not justisfied (1 compound ab initio calculation with minimal GLO basis set uses 1 - 2 hours on NAS computer, performance of 0.25 Cray/XMP).

Models 12 from CNDO results have been finally appeared to be a resonable correlation model and a good compromise between model simplicity, computational time and prediction accuracy.

5.1.4 Prediction of Active Center

A major reason for generating structure - activity correlation equations is to suggest the active site of drugs, where the drug exerts its characteristic effect. Figure 5.3 shows the predicted active center of chloroquine drug that the potential site are atoms in heterocyclic ring N1, C9, C10, C4, N2 and C2 and C3 have additional, but minor influence (Figure 5.3).

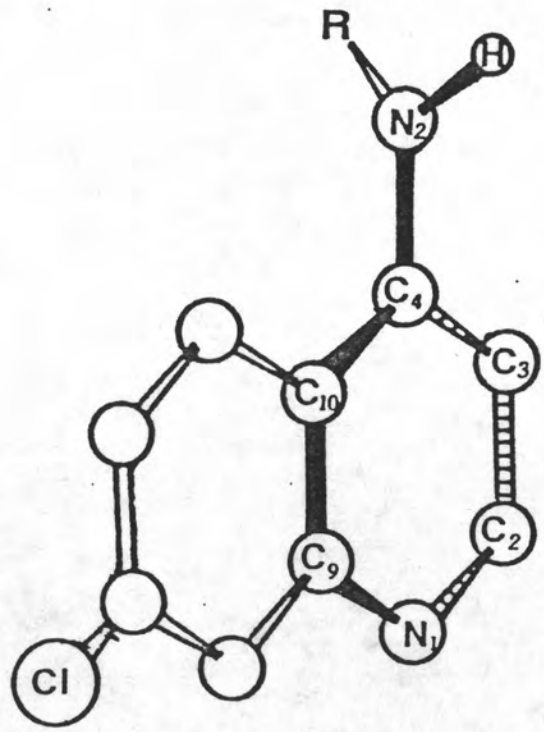


Figure 5.3 Predicted active center of chloroquine drug

The feature as shown in figure 5.3 allow some speculation about the potential receptor molecules for chloroquine drugs. The active center seems to be suitable to bind to nucleic bases, being a hydrogen bond acceptor via N1 and a hydrogen bond donor via N2-H. Once attached to a nucleic base it would prevent the approach of other nucleic bases for pairing through its bulky background groups, provided hydrogen bonding is strong enough. The compounds could be assumed, therefore to interfere with the plasmodia's RNA, binding to its nucleic bases and hindering thus replication. This assumption is backed by the fact that no antimalarial drug of either type is known with two substituents at N2 (and hence not providing a proton for hydrogen bonding).

Not only the substituent and amino side chain that influence their antimalarial potency, but also the number and position of nitrogen atoms in the heterocyclic ring structure and the size of the aromatic ring as reported by O'Brien and Hahn (50).

The influence of the number and position of nitrogen atoms in the heterocyclic ring structure on antimalarial potency is demonstrated by comparing the relative activities of certain quinolines, isoquinolines, quinazolines, and cinnolines (Table 5.1). The quinolines exhibit superior antimalarial properties. This may depend on differences in the electronic configuration of the ring structure that are likely to alter the ring-ring interaction between DNA bases and the drug.

The variation in the size of the aromatic rings of the drugs affect their interaction with DNA as well as their antimalarial potency as shown in table 5.2. Although the planar area of the acridine ring is approximately 40 A^2 (50) and more nearly

commensurate with that of a base pair in DNA than is the area of the quinoline ring, the data show that the acridines possess only 10% of the antimalarial potency of the corresponding 4-aminoquinolines.

Table 5.1 Effect of the number and position of ring nitrogens on antimalarial activity

(R_1 = 1-diethylamino-4-aminopentane:
R_2 = 1-diethylamino-3-aminopropane)

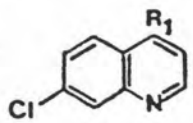
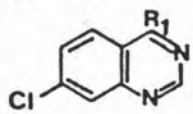
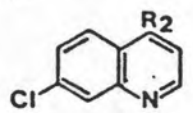
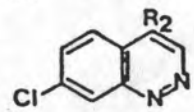
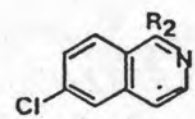
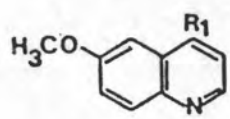
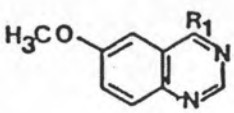
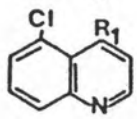
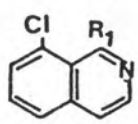
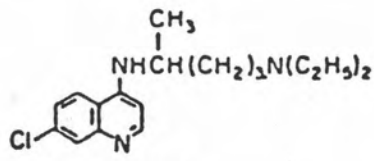
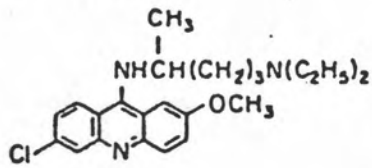
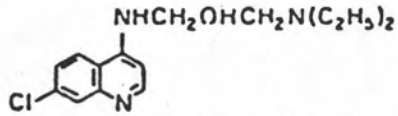
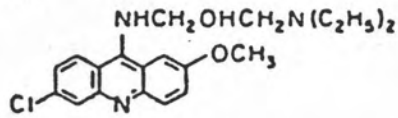
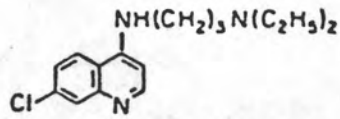
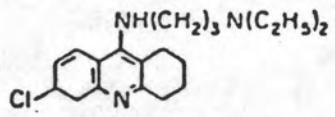
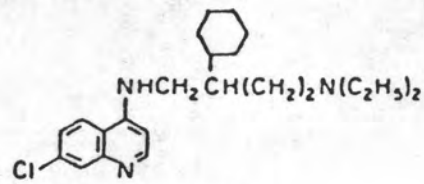
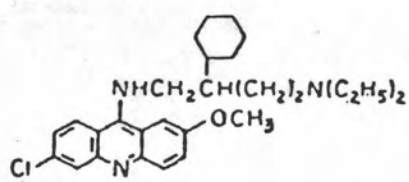
Structure	Antimalarial activity
	100
	13
	80
	2
	0
	10
	2
	3
	0

Table 5.2 Effect on antimalarial activity of increasing the size of the heterocyclic ring

Structure	Antimalarial activity
	100
	12
	100
	10
	80
	9
	50
	5

According to our predicted active center of the privious study (61) and this work (Figure 5.3) and the observation activity of various antimalarial drugs in Tables 5.1 and 5.2, the molecular structure of antimalarial drugs which possess active center contibuting to receptor binding is proposed to consist the following festures:

- quinoline ring
- two nitrogens, one carrying a hydrogen atom
- two nitrogens are linked by aromatic carbon atoms in a "cis" arrangment by 3-4 atoms
- suitable size of planar ring structure
- the background of the active center is formed by bulky groups (aromatic ring system and side chain)

Nevertheless, the correlations which we developed in favor of the validity of the assumption that antimalarial activity in chloroquine analogs is primarily based on the electronic distribution of such drugs in quinoline ring nucleus to interact with receptor in the manner we described.

5.2 Mefloquine Antimalarial Drugs

5.2.1 Electron Distribution in Mefloquine Drug

Consideration of mefloquine molecule we will see that the electron distribution in molecule is similar to chloroquine, i.e. the charges of the heterocyclic ring atomic charges is highly relevant as indicated in Figure 5.4. It is important to note that O and N2 atoms of amino side chain show the

higher influence of net charges than N1 and any other atoms in molecule (Figure 5.4). The changes of these atoms are therefore included in our testing models

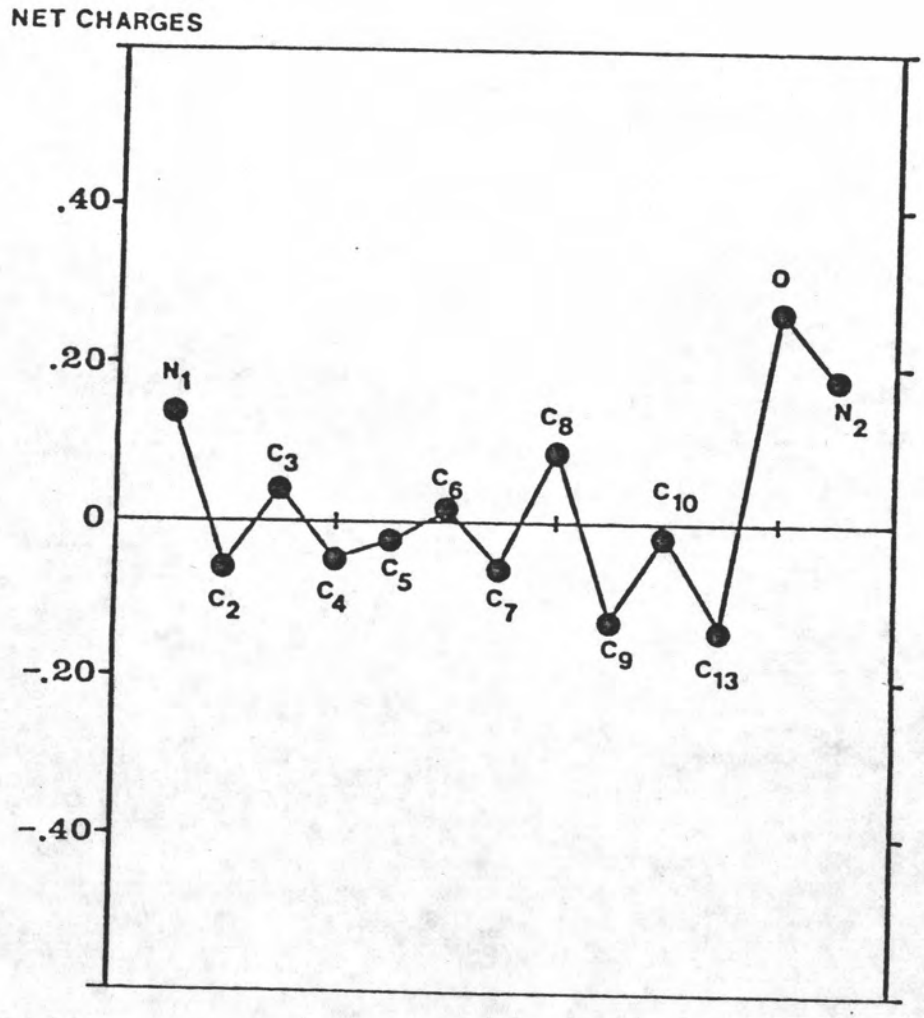


Figure 5.4 Net charges at various atoms in quinoline ring, C13, O and N2 of mefloquine

5.2.2 Electron Distribution-Activity Relationships of Mefloquine Antimalarial Drugs According to CNDO/2 Calculation

Electron distribution - activity relationships of the most recent important active antimalarial drugs, mefloquine, has been investigated by the same fitting procedure as chloroquine drugs. Again, the inclusion of all atoms of quinoline ring in the fitting equations (model 0) led to the conclusion that C5-C8 are less influence than other atoms, therefore they are grouped as one variable in the fitting equation in order to decrease the variables as we have mentioned in section 5.1.2.

Linear modelling for mefloquine drugs has shown to be more complicated than the results obtained in the case of chloroquine analogs. The fitting results of all models as given in Tables 4.7a - 4.7d bring up to surprisingly the strong effect of C13, O and N2 over other variables when these atomic net charges are included in equation. From each type of fitting models 7, 15, 21, are the models which give the low Cav and Cmax values and in the acceptable range.

Model 7:

$$\begin{aligned} \ln A = {} & -19.275*qN1 - 38.733*qC2 - 1.712*qC3 + 114.101*qC4 \\ & + 445.422*qC13 + 590.899*qO - 2391.873*qN2 - 6.925*qrest \\ & + 0.488*u + 341.345 \end{aligned} \tag{5.3}$$

SD = 0.729 Cmax = 1.536 N = 21

Model 15:

$$\ln A = 70.519*qN1 - 65.300*qC4 - 21.914*qC9 - 199.239*qC10 + 832.672*qC13 + 65.562*q0 - 304.986*qN2 - 5.070 + 0.325*u + 131.092 \quad (5.4)$$

SD = 0.676 Cmax = 1.462 N=21

Model 21:

$$\ln A = 3.792*qN1 - 45.879*qC2 + 5.723*qC3 - 2.358*qC4 - 0.866*qC9 - 182.393*qC10 + 832.393*qC13 + 150.654*q0 - 1092.737*qN2 + 0.433*u + 243.400 \quad (5.5)$$

SD = 0.639 Cmax = 1.535 N = 21

Model 21 is quite satisfied by its low Cav. However, more investigations have been done by taking C13, O, N2 accompanying by C26 in model 22 - 26, as the main fitting variable. The values of SD and Cmax are not improved but it seems that model 26 is a preferential model for predicting an active site which will be discussed in the next section.

Model 26:

$$\ln A = 641.157*q0 + 410.946*qC13 + 186.826*qC26 - 2460.031*qN2 + 101.929*qC4 - 19.621*qN1 - 6.916*qrest + 0.507*u + 349.574 \quad (5.6)$$

SD = 0.725 Cmax = 1.537 N = 21

The plot of calculated versus observed antimalarial activity (ln A) for model 26 is presented in Figure 5.5a.

Figure 5.5b shows the plot of $\ln A_{obs} - \ln A_{calc}$ or $\Delta \ln A$ versus $\ln A_{cal}$ for model 26.

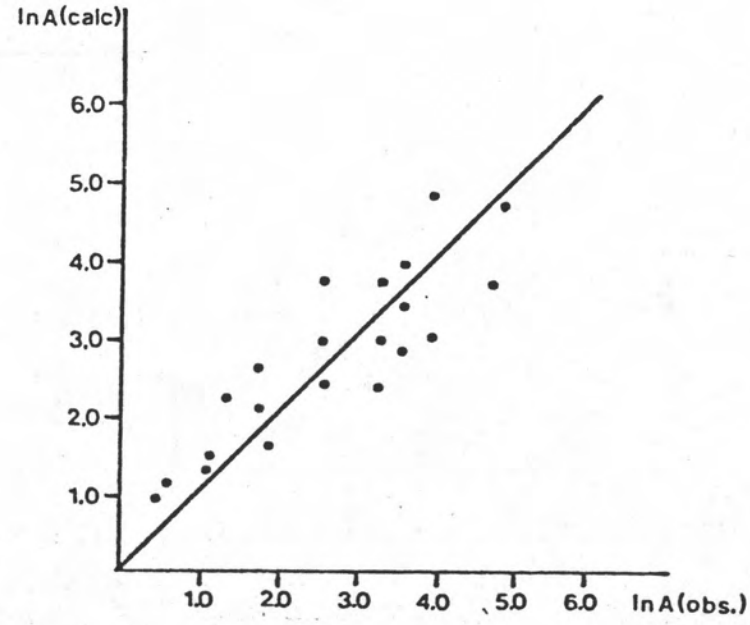


Figure 5.5a The plot of calculated versus observed antimalarial activity (lnA) for model 26

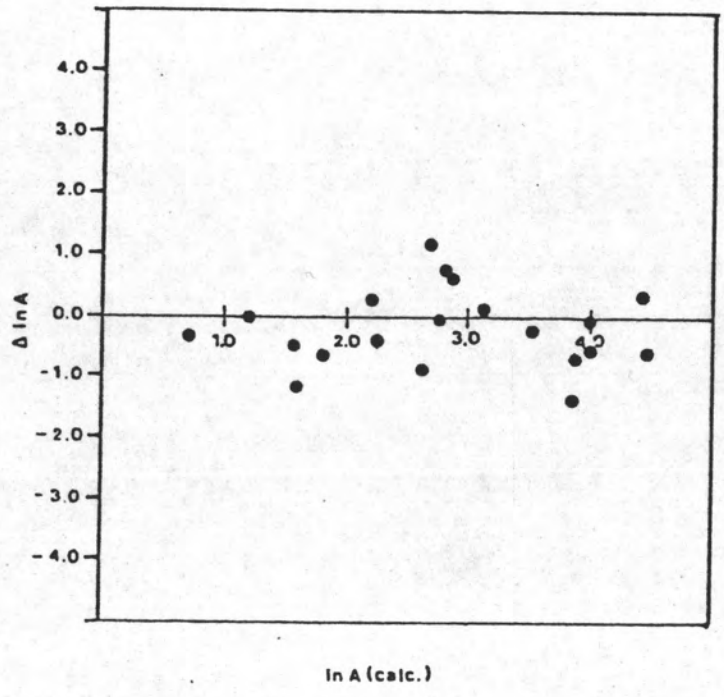


Figure 5.5b The plot of Δln A versus ln A_{cal} for model 26

5.2.3 Prediction of Active Center

The ability to take into consideration the active center of mefloquine drugs is critical for generating a meaningful correlation equations. However, the large parameter size of the atomic charges of atoms N2, O, C13 and C26 indicate that these atoms may form part of the active center of mefloquine (Figure 5.6).

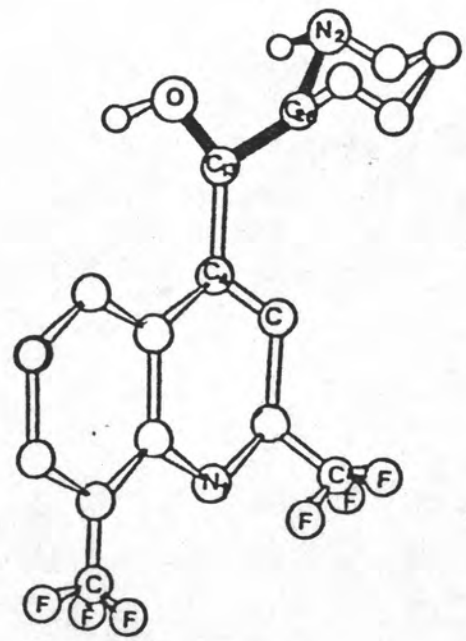
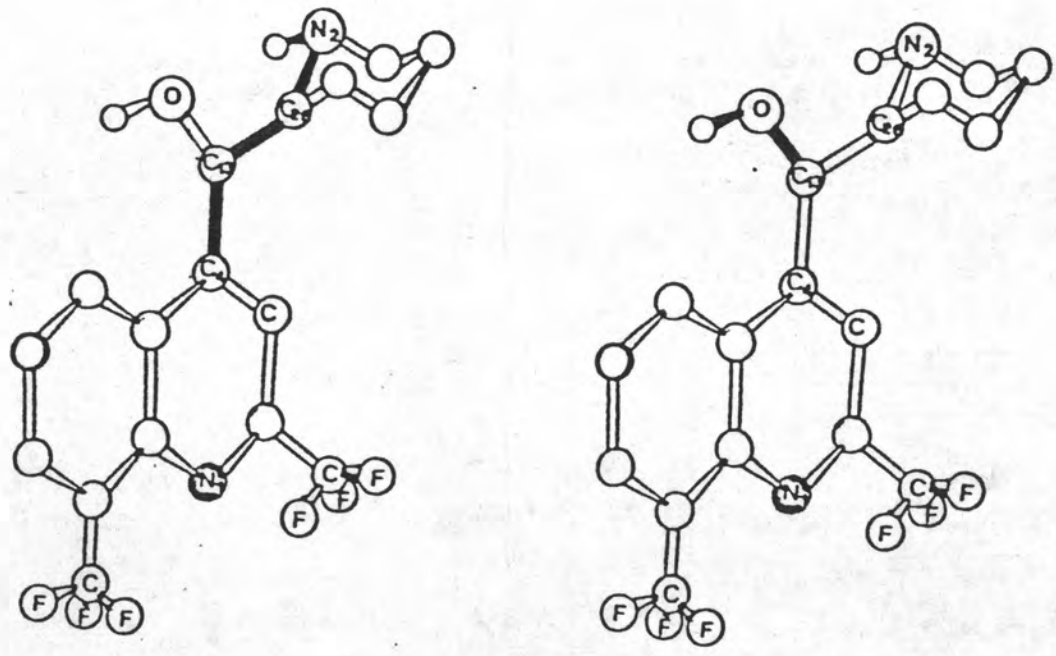


Figure 5.6 Predicted active center of mefloquine drug

As in chloroquines case, this active center composes of H-bond acceptor and H-bond donor as binding site to nucleic base via O and N2-H, respectively. The difference from chloroquine is the active center not being in quinoline ring. From experimental observation, the mode of mefloquine's antimalarial activity is thought to be differ from those suggested for chloroquine (62), i.e. mefloquines does not intercalate or otherwise strongly interact with

DNA (63,64).

Other possibilities for the active center can be from consideration of mefloquine structure and from our discussions on the drug - receptor interaction through H-bond. These are shown in Figures 5.7a and 5.7b. The distance between H-bond acceptor and H-bond donor for intercalation must be calculated in order to get the best possibility of prediction. This is beyond our scope but should be useful for future work.



a. b.

Figure 5.7 The two possibilities of an active center of mefloquine

a. The hydrogen bond acceptor via N1 and hydrogen bond donor via N2

b. The hydrogen bond acceptor via N1 and a hydrogen bond donor via O-H