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Mechanism of Antimalarial Activity of Chloroquine Analogs from Quantitative Structure–Activity Studies. Free Energy Related Model[1a,b]

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The antimalarial activities of chloroquine and 32 of its analogs have been subjected to regression analyses using a free energy related structure–activity model designed in part to test the DNA intercalation mechanism of action proposed by O'Brien and Hahn. Molecular parameters included in the analyses are the OctOH–H_2O partition coefficients and the charges on the 7 substituent of the quinoline ring and the two N of the 4-diamino side chain. The partition coefficients were calcd from substituent values in the literature and the charges were obtained by combining Hückel and Del Re MO charge distributions. The statistical quantities, R^2, F (overall), and explained variance, were calcd for each regression analysis to assess goodness of fit. Under restrictions consistent with the O'Brien–Hahn model, a reasonably high level of structure–activity correlation can be obtained. The importance of the charge and size of the 7 substituent and the separation between the two nitrogens of the 4-diamino side chain was verified. The results suggest that the hydrophobic or steric properties of the terminal amine group of the 4 side chain should be considered along with the charge on N. No high level of correlation was found when structural variations were allowed at more than one substituent position on the quinoline ring. This suggests that the role to be played by substituents at one ring position is influenced appreciably by the nature of substituents at other ring positions.

Those interested in drug design are beginning to turn their attention more and more to the development and utilization of quantitative structure–activity relationships in order to gain insight into the modes of action of drugs. The availability of a large volume of antimalarial activity data on extensive series of molecules spanning several general classes of potential antimalarial agents makes this a very attractive area for continuing and expanding these efforts. The series of chloroquine analogs is particularly well suited to this purpose in view of the existence of a proposed detailed mode of action for these molecules as antimalarials. One may use the quantitative structure–activity studies, on the one hand, to "test" the proposed model, and, on the other, assuming the model to be basically correct, to attempt to sharpen or modify the details of the model.

O'Brien and Hahn[2a] have offered a model to account for the antimalarial activity of chloroquine and its congeners (Figure 1). In particular, they suggested that: (1) these compounds exert their antimalarial effect by intercalation with the parasite DNA, and that the activity of a given compound depends on the stability of its complex with DNA; (2) high activity requires an electronegative group attached to position 7 of the quinoline ring; (3) the diamino side chain attached to the quinoline ring at position 4 bridges the two DNA strands by electrostatic interactions between the diamino nitrogens and the DNA phosphate groups; and (4) substitution of any other groups at any other ring position, except position 6 which is almost equivalent to position 7 for this model, would be expected to alter binding to DNA and thus diminish activity.

To support this model, considerable evidence derived from *in vitro* intercalation studies,[2b] *in vivo* bactericidal studies,[3] and antimalarial activity data selected[2a] from the literature[4,5] was offered. The first of these studies demonstrated that chloroquine can intercalate with DNA, and the second, that, at least for the strain of *Bacillus megaterium* studied, chloroquine inhibits DNA (and at higher concentrations, RNA) synthesis.

It is primarily from the third study, the antimalarial activities of chloroquine and its congeners, that O'Brien and Hahn deduced the roles to be played by the substituents attached to position 7 of the ring and the diamino side chain attached to position 4 (here, *in vitro* studies[6] on the binding of aliphatic diamines to DNA were also helpful). Their observations, based on general trends in the activity data, were qualitative in nature. The results presented here represent an attempt to evaluate quantitatively these trends and to test some of the features of the model. The structure–activity equation designed for this purpose is discussed in the next section.

Structure–Activity Equation.—It has been assumed that the interaction of the drug D (chloroquine, etc.) with the biological substrate S (DNA) may be represented as

$$D + S \underset{}{\overset{K}{\rightleftharpoons}} D\text{–}S \xrightarrow{k} P$$

where K is the equilibrium constant for the formation of the drug–substrate complex and k is the rate constant

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(2) (a) R. L. O'Brien and F. E. Hahn, *Antimicrob. Ag. Chemother.*, 315 (1965). (b) R. L. O'Brien, J. L. Allison, and F. E. Hahn, *Biochim. Biophys. Acta*, **129**, 622 (1966).

(3) F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Olenick, *Mil. Med.*, **131**, 1071 (1966).

(4) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents. Chemotherapy of *Plasmodium gallinaceum* Infections; Toxicity; Correlation of Structure and Action," Public Monograph No. 9, 1953, pp 64–74.

(5) F. Y. Wiselogle, Ed., "A Survey of Antimalarial Drugs 1941–1945," Vol. II, Part II, J. W. Edwards, Ann Arbor, Mich., 1946, pp 1112–1164.

(6) H. R. Mahler and B. D. Mehrotra, *Biochim. Biophys. Acta*, **68**, 211 (1963).

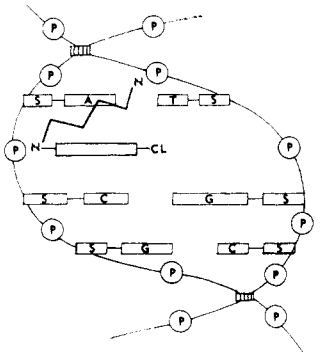


Figure 1.—Proposed structure of DNA–chloroquine complex consistent with model of O'Brien and Hahn.[2a]

for the conversion of this complex into product P. The rate of formation of product can be written as

$$\frac{d[P]}{dt} = k[D\text{–}S] \quad (1)$$

For the equilibrium constant K one has, assuming a steady-state concentration of complex,

$$K = \frac{[D\text{–}S]}{[D][S]} = \exp(-\Delta G/RT) \quad (2)$$

where ΔG is the free energy of formation of D–S from D and S, R is the gas constant, and T is the absolute temperature. Solving eq 2 for [D–S] and substituting in eq 1 gives

$$\frac{d[P]}{dt} = k[D][S]\exp(-\Delta G/RT) \quad (3)$$

Here, [D] represents the concentration of the drug in the vicinity of the substrate and can be assumed to be quite different from the administered concentration or dose, $[D_0]$. It is this latter quantity which is usually tabulated by the experimentalist while the former remains essentially unknown. To circumvent this difficulty, it is usually assumed that

$$[D] = A[D_0] \quad (4)$$

where A is less than unity. Hansch and coworkers[7] have related the factor A to the OctOH–H_2O partition coefficient of the drug, using the relationship

$$A = \alpha \exp[-(\pi - \pi^\circ)^2/\beta] \quad (5)$$

where π is the logarithm of the partition coefficient, and α, β, and π° are constants. Substitution of eq 4 and 5 into eq 3 and expansion of ΔG as $\Delta E + P\Delta V - T\Delta S$ gives

$$\frac{d[P]}{dt} = k\alpha[D_0][S]\exp[-(\pi - \pi^\circ)^2/\beta]\exp[-\Delta E/RT] \times \exp[-(P\Delta V - T\Delta S)/RT] \quad (6)$$

If one assumes that the drug–substrate complex is stabilized primarily by electrostatic interactions and, further, that these can be approximated using the simple coulomb potential with D and S represented as systems of point charges, the stabilization energy, ΔE, can be evaluated as

$$\Delta E = \Sigma_i\Sigma_j \frac{Q_i^D Q_j^S}{r_{ij}} = \Sigma_i Q_i^D \Sigma_j \frac{Q_j^S}{r_{ij}} \quad (7)$$

where Q_i^D is the charge at point (atom) i of the drug, Q_j^S is the charge at point (atom) j of the substrate, and r_{ij} is the distance separating these two points. Substitution of eq 7 into eq 6 leads to

$$\frac{d[P]}{dt} = k\alpha[D_0][S]\exp[-(\pi - \pi^\circ)^2/\beta] \times \exp[-\Sigma_i\Sigma_j Q_i^D Q_j^S/RTr_{ij}] \times \exp[-(P\Delta V - T\Delta S)/RT] \quad (8)$$

Assuming all terms on the right side of eq 8 to be independent of [P] and t, integration from zero time and product to some particular values P^* and t^* gives

$$P^* = k\alpha[D_0^*][S]\exp[-(\pi - \pi^\circ)^2/\beta] \times \exp[-\Sigma_i\Sigma_j Q_i^D Q_j^S/RTr_{ij}] \times \exp[-(P\Delta V - T\Delta S)] \quad (9)$$

where $[D_0^*]$ is the drug dosage required to produce $[P^*]$ in time t^*. Thus, $[D_0^*]$ corresponds to such commonly reported quantities as LD_{50}, ED_{50}, TIC4, etc. Taking the log of each side and rearranging one may obtain

$$\log \frac{1}{[D_0^*]} = -\frac{2.303}{\beta}\pi^2 + \frac{4.606}{\beta}\pi^\circ\pi - \frac{2.303}{\beta}\pi^{\circ 2} - \Sigma_i Q_i^D\left(\frac{\Sigma_j Q_j^S}{RTr_{ij}}\right) + \log(k\alpha[S]t^*/P^*) - 2.303(P\Delta V - T\Delta S) \quad (10)$$

This equation is to be used to study *variations* in the activities of a series of very similar molecules assumed to be active *via* the same mechanism. For this reason, all quantities in eq 10 which are assumed not to vary from one member of the series to the next can be treated as simple constants. The last two terms of the equation and the parameters β and π° can be so treated. Further, if one assumes that each of the drug molecules is oriented relative to the substrate in exactly the same manner, one can also treat the quantity $(\Sigma_j Q_j^S/RTr_{ij})$ as a constant for a given value of the index i. Since O'Brien and Hahn developed their model in terms of electrostatic interactions involving the substituent attached to position 7 and the two nitrogens of the diamino side chain at position 4, interactions involving all other point charges of the drug molecule will be considered constant also. Under these conditions, eq 10 can be rewritten as

$$\log \frac{1}{[D_0^*]} = a\pi^2 + b\pi + cQ_x + dQ_{N1} + eQ_{N_2^+} + f \quad (11)$$

where Q_x is the charge on the substituent attached to ring position 7, Q_{N1} is the charge on the diamino N at ring position 4 and $Q_{N_2^+}$ is the charge on the terminal N of the diamino side chain (assumed to be protonated). The coefficients and constant have been written simply as a, b, . . . f.

(7) C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, **86**, 1616 (1964).

From other sources, one may obtain values for $[D_0^*]$, π, Q_x, Q_{N1}, and Q_{N2}^+ for each of the drugs in the series. Then, using the techniques of regression analysis, the coefficients $a, b, \ldots, f$ can be evaluated, their significance tested, and the ability of the activity equation to explain the observed variation in the biological data measured (these aspects are discussed later).

The parameter π is usually interpreted in terms of transport and/or hydrophobic bonding properties of the molecule.[7,8] Accordingly, a high degree of activity correlation with π would imply that ability of the drug to move from the point of application (crossing phase boundaries such as membranes, etc.) and possibly interact with the substrate *via* hydrophobic bonding is important. Activity dependence on the parameters Q_X, Q_{N1}, and Q_{N2}^+ should provide a test of several features of the model proposed by O'Brien and Hahn.

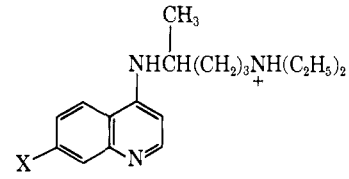
It must be recognized, however, that steric requirements also are implied by the intercalation model.[2a] No such factors have been incorporated in the activity equation (eq 11). Similarly, no explicit allowance has been made for effects due to substituents attached at ring positions other than 4 and 7. These factors must be taken into consideration in the interpretation of the results of the regression analyses.

Calculation Procedures.—The antimalarial activity data used in this study were selected from that listed by O'Brien and Hahn.[2a] The quantity $1/[D_0^*]$ (eq 11) was taken to be 0.1/METD where METD (the minimum effective therapeutic dose) is the dose required to reduce the parasitemia in White Rock chicks infected with *Plasmodium gallinaceum* to less than 25% of controls.[4,5] The experimental error inherent in these activity data will, of course, contribute to uncertainty in the results. For example, since the METD were determined by administering drug dosages in a geometric progression with base **2**[4,5] a drug with an METD of 0.050 is not necessarily appreciably more or less active than one with an METD of 0.025 or 0.100, respectively.[5]

The charges, Q_X, Q_{N1}, and Q_{N2}^+, were obtained by combining the results of Hückel π electron calculations[9] and Del Re σ electron calculations.[10] For both calculations, parameter values recommended to reproduce dipole moments were used.[9,11] (Del Re parameters used for Br are those reported by Bass[12]). Computer programs for the Del Re and Hückel calculations were written by G. E. Bass and K. Sundarum, respectively. Values for the parameter π were calculated as the sum of substituent constants, π, gleaned from the publications of Hansch and coworkers.[7,8,13–17]

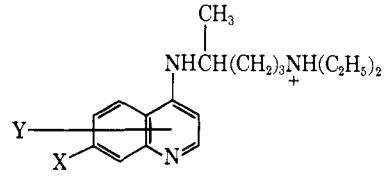
In the analyses reported here, only compounds selected from the series listed by O'Brien and Hahn have been considered. These can be divided into three series.

Series I is composed of compounds which differ from chloroquine only in the substituent attached to position 7 of the ring.



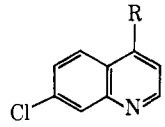
X = substituent group

Series II is composed of compounds which differ from chloroquine in the substituents attached to position 7 and one other position but not at position 4.



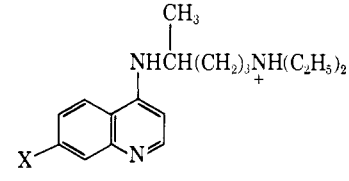
X, Y = substituent groups

Series III is composed of compounds which differ from chloroquine only in the diamino side chain attached at position 4. The molecules included in each of these series and corresponding parameter values are given in Tables I–III.



R = diamino side chain, terminal nitrogen protonated

TABLE I
ANTIMALARIAL ACTIVITIES AND PARAMETER VALUES FOR SERIES I



X	Activity[a]	π^b	Q_X	Q_{N1}	Q_{N2}^+
Cl	100	4.20	−0.124	−0.264	0.525
I	67	4.71	−0.101	−0.264	0.525
Br	50	4.35	−0.109	−0.264	0.525
F	50	3.63	−0.176	−0.264	0.525
CF_3	50	4.65	−0.042	−0.264	0.525
OCH_3	14	3.47	−0.071	−0.264	0.525
CH_3	7	3.99	−0.026	−0.264	0.525
H	7	3.49	+0.053	−0.264	0.525

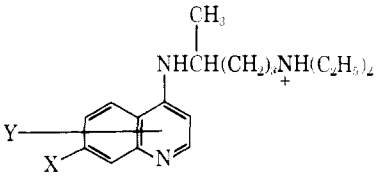
[a] Activities are relative to chloroquine which is 100. (R. L. O'Brien and F. E. Hahn, *Actimicrob. Ag. Chemother.*, 315 (1965). [b] π = sum of Hansch π values of all segments (including the quinoline ring) of the molecule.

For the analyses reported here, the charge on the terminal N of the diamino side chain was always taken to be that of the protonated cation (designated Q_{N2}^+). The N attached to the ring was taken to be formally

(8) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).
(9) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, New York, N. Y., 1963, p 67.
(10) G. Del Re, *J. Chem. Soc.*, 4031 (1958).
(11) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Theor. Chim. Acta*, **8**, 212 (1967).
(12) G. E. Bass, Ph.D. Thesis, Vanderbilt University, Nashville, Tenn., 1970, p 79.
(13) C. Hansch and F. Helmer, *J. Polym. Sci.*, **6**, 3295 (1968).
(14) C. Hansch, J. E. Quinlan, and G. L. Lawrence, *J. Org. Chem.*, **33**, 347 (1968).
(15) F. Helmer, K. Kiehs, and C. Hansch, *Biochemistry*, **7**, 2858 (1968).
(16) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965).
(17) A. Leo, C. Hansch, and C. Church, *ibid.*, **12**, 766 (1969).

TABLE II

ANTIMALARIAL ACTIVITIES AND PARAMETER VALUES FOR SERIES II

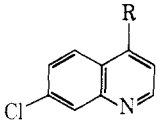


X	Y	Activity[a]	π[b]	Q_X	Q_{N1}	Q_{N2^+}	Q_Y
Cl	H	100	4.20	−0.124	−0.264	+0.525	+0.055
H	6-Cl	100	4.20	+0.055	−0.264	+0.525	−0.126
H	5-Cl	3	4.20	+0.053	−0.264	+0.525	−0.122
H	8-Cl	3	4.20	+0.055	−0.264	+0.525	−0.124
H	3-Br	3	4.35	+0.053	−0.261	+0.525	−0.115
H	6-OCH_3	10	3.47	−0.213	−0.266	+0.525	−0.213
Cl	6-CH_3	25	4.70	−0.125	−0.264	+0.525	−0.104
Cl	3-CH_3	15	4.70	−0.124	−0.264	+0.525	−0.092
Cl	2-CH_3	10	4.70	−0.124	−0.264	+0.525	−0.098
Cl	2-Ph	2	6.33	−0.124	−0.265	+0.525	−0.027
Cl	3-Br	6	5.06	−0.124	−0.262	+0.525	−0.113
Cl	5-Br	5	5.06	−0.120	−0.269	+0.525	−0.105
Cl	8-NH_2	2	2.97	−0.127	−0.265	+0.525	−0.443

[a] See footnote *a*, Table I. [b] π = sum of Hansch π values of all segments (including the quinoline ring) of the molecule.

TABLE III

ANTIMALARIAL ACTIVITIES AND PARAMETER VALUES FOR SERIES III



R	Activity[a]	π[b]	Q_X	Q_{N1}	Q_{N2}	Q_{N2^+}
$NHCH(CH_3)(CH_2)_3N^+(C_2H_5)_2H$	100	4.20	−0.124	−0.264	−0.205	+0.525
$NHC_6H_{10}N^+(C_2H_5)_2H$[c]	100	4.18	−0.124	−0.264	−0.208	+0.524
$NHC_6H_{10}N^+(C_4H_9)_2H$[c]	25	6.26	−0.124	−0.264	−0.208	+0.524
$NHC_6H_{10}N^+H_2C_2H_5$[c]	100	3.25	−0.124	−0.264	−0.359	+0.346
$NHC_6H_{10}N^+H_2CH(CH_3)CH_3$[c]	50	3.64	−0.124	−0.264	−0.361	+0.344
$NHC_6H_{10}N^+H_2C_6H_{11}$ *cis*	50	4.66	−0.124	−0.264	−0.361	+0.348
$NHC_6H_{10}N^+H_2C_6H_{11}$ *trans*	25	4.54	−0.124	−0.264	−0.361	+0.348
$NHCH(CH_3)(CH_2)_3N^+H_2CH_3$	100	2.75	−0.124	−0.264	−0.355	+0.349
$NHCH(CH_3)(CH_2)_3N^+H_2C_2H_5$	50	3.27	−0.124	−0.264	−0.357	+0.347
$NH(CH_2)_3N^+(C_2H_5)_2H$	80	3.28	−0.124	−0.262	−0.209	+0.526
$NH(CH_2)_3N^+(CH_2CH_2OH)_2H$	8	−0.32	−0.124	−0.262	−0.205	+0.527
$NH(CH_2)_3N^+(C_6H_{13})_2H$	6	7.44	−0.124	−0.262	−0.210	+0.525
$NH(CH_2)_3N^+(C_8H_{17})_2H$	5	9.52	−0.124	−0.262	−0.210	+0.525
$NHCH_2CH(OH)CH_2N^+(C_2H_5)_2H$	100	0.96	−0.124	−0.261	−0.208	+0.527

[a] See footnote *a*, Table I. [b] π = sum of Hansch π values of all segments (including the quinoline ring) of the molecule. [c] C_6H_{10} is cyclohexane substituted in the 1 and 4 positions.

neutral. Analyses not reported here[18] served to indicate that, at least for the terminal N, it makes little difference in the extent of correlation whether the nitrogen is considered protonated or not.

As a measure of the "goodness of fit" for a particular activity equation, the square of the multiple correlation coefficient, R^2 (R^2 = 1.0 indicates perfect correlation), the overall F ratio for the regression and corresponding significance level, and the amount of explained variance, eV, were calculated.[19] The regression coefficient gives an indication of the degree of correspondence between the experimentally observed antimalarial activities and those calculated with the trial linear equation resulting from the regression analysis. R^2 is interpreted as the fraction of the sum of squares of the deviations of observed activities from the mean activity that is attributed to the regression. The F ratio is the decision statistic of the F test of significance. The overall F test with this model is a test of the null hypothesis that all of the parameter coefficients are equal to zero; in other words, the mean activity would be as good an estimate of the true activity as the activity calculated from the linear regression equation if the null hypothesis is true. The explained variance gives the fraction of the variance of the antimalarial activities which is attributed to the linear relationship of those parameters included in the analysis. Even though regression coefficients may prove to be statistically significant with the F test, it is not uncommon to find that the fraction of explained variance is quite small. This would indicate that most of the variance in the activities must be attributed to variables not included in the regression. It should be mentioned that while an explained variance of 1.0 indicates perfect correlation, it is possible for the calculated

(18) G. E. Bass, D. R. Hudson, J. E. Parker, and W. P. Purcell, "Progress in Molecular and Subcellular Biology," Vol. 2, Springer-Verlag, Berlin, in press.

(19) G. W. Snedecor and W. G. Cochran, "Statistical Methods," Iowa State University Press, Ames, Iowa, 1967, pp 386–388, 400–402.

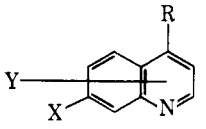
explained variance to be negative. This occurs when, on a per degree of freedom basis, the mean activity is a better approximation to the true activity than the calculated activity.

The regression analyses were carried out using a computer program developed in this laboratory based on IBM 1620 Program 06.0.148, "Single and Multiple Linear Regression Analyses." These and the molecular orbital calculations reported here were performed on an IBM 1620 computer.

Analyses Involving Series I, II, and III Combined.—The overall ability of the structure–activity equation (eq 11) to explain variations in antimalarial activity was tested by carrying out regression analyses on the combined series of 33 molecules comprising series I–III. The combinations of parameters examined and results obtained are presented in Table IV. In particular, it

TABLE IV

REGRESSION ANALYSES RESULTS FOR SERIES I, II, AND III COMBINED



$f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}^+)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.08	85	0.05
$a\pi^2 + b$	0.11	90	0.08
$a\pi^2 + b\pi + c$	0.11	80	0.05
$aQ_X + b$	0.00	<25	−0.03
$aQ_{N1} + b$	0.01	25	−0.03
$aQ_{N2}^+ + b$	0.16	97.5	0.13
$aQ_X + bQ_{N1} + cQ_{N2}^+ + d$	0.17	85	0.09
$a\pi^2 + b\pi + cQ_X + dQ_{N1} + eQ_{N2}^+ + f$	0.25	80	0.11

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}^+)$.

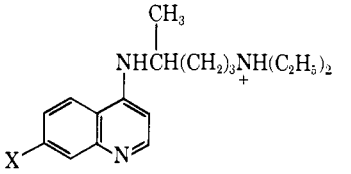
is seen that the Hansch parameter can explain only 5–8% of the variance. In some cases, this behavior might be interpreted as an implication that a stereospecific drug–substrate interaction is involved and/or that transport of the drug to the active site is not a controlling factor within the series.[7,8] Further, electrostatic interactions involving Q_X, Q_{N1}, and Q_{N2}^+ also do not appear to be able to account for the activity variations. This implies that if the model of O'Brien and Hahn is assumed to be correct, the reservations allowed there for the effect of ring substituents at positions other than 4 and 7 and for steric effects must be of substantial importance.

Taken all together, the results in Table IV indicate that the structure–activity equation developed here cannot explain the variations in the biological data of this series of molecules. This is not to say that the model of O'Brien and Hahn is wrong, for, as pointed out above, important steric aspects of the model are not reflected in the structure–activity equation. If one analyzes series I–III individually, however, these factors can be isolated and their possible importance investigated.

Analyses Involving Series I.—In this series, substituent variation occurs only at ring position 7. Thus, no complications are introduced as a result of changes in the diamino side chain or the presence of additional ring substituent variations. Examination of Table I reveals that Q_{N1} and Q_{N2}^+ do not vary appreciably in this series and thus need not be included in the regression analyses. The parameter combinations considered and results obtained for this series of eight molecules are presented in Table V.

TABLE V

REGRESSION ANALYSES RESULTS FOR SERIES I



$f(\pi^2, \pi, Q_X)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.40	90	0.30
$a\pi^2 + b$	0.39	90	0.29
$a\pi^2 + b\pi + c$	0.40	70	0.16
$aQ_X + b$	0.00	<25	−0.16
$a\pi + bQ_X + c$	0.45	75	0.23
$a\pi^2 + bQ_X + c$	0.45	75	0.23
$a\pi^2 + b\pi + cQ_X + d$	0.45	50	0.04

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X)$.

It is seen that the Hansch parameter (as π or π^2) can account for 30% of the variance in the biological data. Taken in conjunction with the relatively high significance level (90%), this implies that, within this series of molecules, transport and/or hydrophobic interactions may be a factor, though only a secondary one.

The complete lack of correlation, significance, and explained variance obtained with Q_X alone is, at first glance, somewhat surprising. One of the basic tenets of the model of O'Brien and Hahn is that, other things being equal, activity should vary with the electronegativity of the substituent attached at position 7 (here the charge calculated for that substituent has been substituted for electronegativity). It must be recalled, however, that this portion of the molecule must be capable of being inserted (intercalated) between the DNA base pairs and that a steric factor is thus implicit in the model. If a substituent is sufficiently large and bulky, it would tend to inhibit intercalation regardless of its electronegativity or charge. In addition, it might be argued that for a polyatomic group, such as CF_3, taking Q_X to be simply the charge on that atom of the group directly attached to the ring (here, C) is unrealistic. Accordingly, the net charge on the group, Q_X (net), was also calculated.

To investigate these possibilities, regression analyses using the relationships

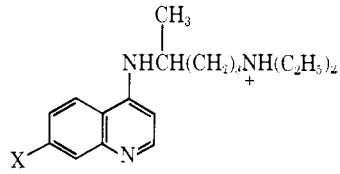
$$\log (0.1/\text{METD}) = aQ_X + b \quad (12)$$

$$\log (0.1/\text{METD}) = aQ_X(\text{net}) + b \quad (13)$$

were carried out starting with the three substituents H, F, and Cl and expanding the series, adding larger and larger substituents one at a time, to include all eight substituents of series I. The results are presented in Table VI.

Inspection of Table VI reveals that R^2 and explained variance both reach a maximum when the largest substituent included is Br, regardless of whether Q_X or Q_X(net) is used. When Q_X is used, the explained variance decreases drastically as the larger, polyatomic

TABLE VI
SEQUENTIAL REGRESSION ANALYSES ON SERIES I

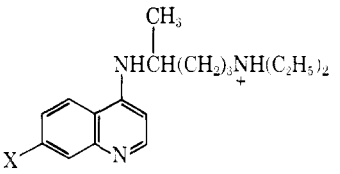


Substituents included	R^2	Significance level, %	eV
Log (0.1/METD) = $aQ_X + b$			
H, F, Cl	0.79	65	0.59
H, F, Cl, Br	0.80	85	0.69
H, F, Cl, Br, I	0.77	90	0.69
H, F, Cl, Br, CH_3	0.44	75	0.25
H, F, Cl, Br, I, CH_3	0.41	80	0.26
H, F, Cl, Br, I, CH_3, CF_3	0.00	<25	−0.20
H, F, Cl, Br, I, CH_3, CF_3, OCH_3	0.00	<25	−0.16
Log (0.1/METD) = aQ_X(net) + b			
H, F, Cl	0.79	65	0.59
H, F, Cl, Br	0.80	85	0.69
H, F, Cl, Br, I	0.77	90	0.69
H, F, Cl, Br, CH_3	0.75	90	0.67
H, F, Cl, Br, I, CH_3	0.73	95	0.66
H, F, Cl, Br, I, CH_3, CF_3	0.63	95	0.55
H, F, Cl, Br, I, CH_3, CF_3, OCH_3	0.59	95	0.52

groups are included. This decrease is much more gradual when Q_X(net) is used. Both of these sets of results indicate that variations in the charge on the substituent can account reasonably well (69% explained variance, 90% significance level) for activity variations as long as the substituents are relatively small. As substituents larger than Br are considered, however, the explained variance decreases, implying that additional variables must be sought to explain the observed biological activities. In the model of O'Brien and Hahn, such an additional variable would be steric inhibition of intercalation.

In view of these findings, the regression analyses incorporating π and π^2 also were repeated for the reduced series with substituents H, F, Cl, Br, and I (Table VII). It is seen that up to 84% of the variance of the activity data can be explained by using Q_X and either π^2 or π.

TABLE VII
REGRESSION ANALYSES INVOLVING SERIES I WITH SUBSTITUENTS H, F, Cl, Br, AND I ONLY



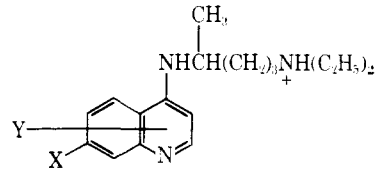
$f(\pi^2, \pi, Q_X)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.46	75	0.28
$a\pi^2 + b$	0.44	75	0.25
$a\pi^2 + b\pi + c$	0.69	65	0.38
$aQ_X + b$	0.77	90	0.69
$a\pi + bQ_X + c$	0.92	90	0.84
$a\pi^2 + bQ_X + c$	0.92	90	0.84
$a\pi^2 + b\pi + cQ_X + d$	0.95	65	0.80

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X)$.

Thus, regression analyses on series I indicate that the charge associated with the substituent attached at position 7, whether taken as Q_X or Q_X(net) can be correlated successfully with antimalarial activity as long as the substituents are not relatively large and bulky. Further, transport and/or hydrophobic binding, as measured by the Hansch parameter, appear to be able to play only a minor role. These findings are consistent with the model of O'Brien and Hahn.

Analyses Involving Series II.—Series II is composed of molecules which have substituents attached to the quinoline ring at positions other than 4 and 7, and which have no variation in the diamino side chain. The parameter combinations considered and results obtained are presented in Table VIII. The parameter

TABLE VIII
REGRESSION ANALYSES INVOLVING SERIES II



$f(\pi^2, \pi, Q_X, Q_Y, Q_{N1}, Q_{N2}{}^+)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.01	25	−0.08
$a\pi^2 + b\pi + c$	0.23	70	0.07
$aQ_X + b$	0.01	<25	−0.08
$aQ_Y + b$	0.13	75	0.05
$aQ_X + bQ_Y + c$	0.14	50	−0.03
$a\pi^2 + b\pi + cQ_X + d$	0.30	60	0.07
$a\pi^2 + b\pi + cQ_X + dQ_{N1} + eQ_{N2}{}^+ + f$	0.03	<25	−0.66
$a\pi^2 + b\pi + cQ_X + dQ_Y + eQ_{N1} + fQ_{N2}{}^+ + g$	0.47	40	−0.06

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X, Q_Y, Q_{N1}, Q_{N2}{}^+)$.

Q_Y is the charge calculated for that atom of substituent Y attached directly to the ring.

The results for this series are remarkable in the virtually complete failure to obtain correlation, coefficient significance, and explained variance. One can only conclude that the addition of the third ring substituent, Y, leads to a drastic deviation from the manner in which the 4,7-disubstituted analogs exert their antimalarial effect. O'Brien and Hahn[2a] suggest that this effect may be due to steric hinderance to intercalation, or an alteration in the relative orientation of the drug to the nucleotide bases which produces a less stable complex than occurs with chloroquine.

The calculations were repeated after eliminating all polyatomic substituents except NH_2 at X and Y. No appreciable improvement was obtained.

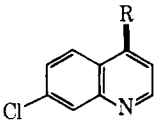
Analyses Involving Series III.—Series III consists of 14 molecules which differ from one another only at the 4-diamino side chain. In the model of O'Brien and Hahn, this side chain is depicted as being electrostatically bound through the diamino nitrogens to phosphate groups on opposite DNA strands. The DNA geometry led O'Brien and Hahn to predict that the effectiveness of this side group should depend on the number of carbons separating the nitrogens; the optimal number for this separation is 4. Nine of the compounds in series III have the two diamino nitrogens

separated by 4 C while, for the remaining 5 compounds, the separation is 3 C.

When all of the molecules in series III were considered together, the only meaningful results were obtained with the Hansch parameter. The combinations of variables tested and results obtained are presented in Table IX.

TABLE IX

REGRESSION ANALYSES RESULTS FOR SERIES III



$f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.24	90	0.17
$a\pi^2 + b$	0.48	99	0.43
$a\pi^2 + b\pi + c$	0.69	99.5	0.63
$aQ_{N1} + b$	0.11	70	0.03
$aQ_{N2}^+ + b$	0.10	70	0.02
$aQ_{N1} + bQ_{N2}^+ + c$	0.13	50	−0.03
$a\pi + bQ_{N1} + c$	0.39	90	0.28
$a\pi + bQ_{N2}^+ + c$	0.29	80	0.17
$a\pi^2 + bQ_{N1} + c$	0.53	97.5	0.45
$a\pi^2 + bQ_{N2}^+ + c$	0.49	95	0.39
$a\pi + bQ_{N1} + cQ_{N2}^+ + d$	0.39	80	0.21
$a\pi^2 + bQ_{N1} + cQ_{N2}^+ + d$	0.54	95	0.40
$a\pi^2 + b\pi + cQ_{N1} + d$	0.70	99	0.61
$a\pi^2 + b\pi + cQ_{N2}^+ + d$	0.70	99	0.61
$a\pi^2 + b\pi + cQ_{N1} + dQ_{N2}^+ + e$	0.70	97.5	0.57

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$.

It is seen that essentially none of the variation in biological activity can be accounted for by using only the charges Q_{N1} and Q_{N2}^+ on the two nitrogens of the side chain. Furthermore, it can be demonstrated (on the basis of F tests) that inclusion of these parameters after π and/or π^2 is statistically unjustified (at the 95% confidence level).

To investigate the possible influence of the separation of the diamino nitrogens, the subgroups with 3- and 4-C separations were analyzed separately. The variable combinations considered and results obtained for the series of 9 molecules with a 4-C separation are presented in Table X. The corresponding information for the series of 5 molecules with a 3-C separation is presented in Table XI.

Examination of Table X reveals that, again, the charges of the nitrogens can account for essentially none of the variance, while π does seem to have some importance. When the regression analyses are carried out using both the Hansch parameter (as π or π^2) and Q_{N2}^+, however, very substantial increases in correlation and explained variance are obtained, suggesting a cooperative effect. In these equations,

$$\log (0.1/\text{METD}) = -0.260\pi + 2.038Q_{N2}^+ + 4.038 \quad (14)$$

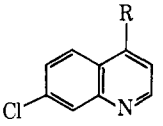
$$\log (0.1/\text{METD}) = -0.285\pi^2 + 2.004Q_{N2}^+ + 3.458 \quad (15)$$

$$\log (0.1/\text{METD}) = -0.013\pi^2 - 0.143\pi + 2.034Q_{N2}^+ + 3.788 \quad (16)$$

the coefficients indicate that activity increases as π decreases and Q_{N2}^+ increases. A possible rationale within the framework of O'Brien and Hahn's model might then be that the size of the alkyl groups on the terminal amine dictates the extent to which the groups can participate in electrostatic binding to a nearby DNA phosphate group.

TABLE X

REGRESSION ANALYSES INVOLVING SERIES III MOLECULES WITH DIAMINO NITROGENS SEPARATED BY 4 C

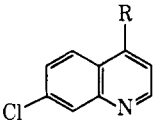


$f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$[a]	R^2	Significance level, %	eV
$a\pi + b$	0.44	90	0.36
$a\pi^2 + b$	0.44	95	0.36
$a\pi^2 + b\pi + c$	0.44	80	0.26
$aQ_{N1} + b$	0.16	70	0.05
$aQ_{N2}^+ + b$	0.01	<25	−0.13
$aQ_{N1} + bQ_{N2}^+ + c$	0.17	40	−0.10
$a\pi + bQ_{N1} + c$	0.45	80	0.26
$a\pi + bQ_{N2}^+ + c$	0.79	99	0.71
$a\pi^2 + bQ_{N1} + c$	0.46	80	0.28
$a\pi^2 + bQ_{N2}^+ + c$	0.78	99	0.71
$a\pi + bQ_{N1} + cQ_{N2}^+ + d$	0.81	95	0.70
$a\pi^2 + bQ_{N1} + cQ_{N2}^+ + d$	0.79	95	0.67
$a\pi^2 + b\pi + cQ_{N1} + d$	0.46	60	0.13
$a\pi^2 + b\pi + cQ_{N2}^+ + d$	0.79	95	0.66
$a\pi^2 + b\pi + cQ_{N1} + dQ_{N2}^+ + e$	0.81	90	0.63

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$.

TABLE XI

REGRESSION ANALYSES INVOLVING SERIES III MOLECULES WITH DIAMINO NITROGENS SEPARATED BY 3 C



$f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$	R^2	Significance level, %	eV
$a\pi + b$	0.30	60	0.07
$a\pi^2 + b$	0.46	75	0.28
$a\pi^2 + b\pi + c$	0.64	55	0.28
$aQ_{N1} + b$	0.42	75	0.23
$aQ_{N2}^+ + b$	0.02	<25	−0.30
$aQ_{N1} + bQ_{N2}^+ + c$	0.42	40	−0.15
$a\pi + bQ_{N1} + c$	0.51	50	0.03
$a\pi + bQ_{N2}^+ + c$	0.81	75	0.62
$a\pi^2 + bQ_{N1} + c$	0.62	55	0.24
$a\pi^2 + bQ_{N2}^+ + c$	0.73	70	0.47
$a\pi + bQ_{N1} + cQ_{N2}^+ + d$	0.93	60	0.73
$a\pi^2 + bQ_{N1} + cQ_{N2}^+ + d$	0.89	50	0.56
$a\pi^2 + b\pi + cQ_{N1} + d$	0.82	45	0.28
$a\pi^2 + b\pi + cQ_{N2}^+ + d$	0.94	60	0.76

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_{N1}, Q_{N2}^+)$.

The results in Table XI for the 5-molecule subgroup in which the diamino nitrogens are separated by 3 carbons, though understandably less significant, generally parallel those for the subgroup with 4-C separations. No support is found, however, for the contention that the correlation of π and Q_{N2}^+ reflects a cooperative effect; here, activity increases as both π and Q_{N2}^+ decrease. The overall significance level for this calculation, however, is only 75%.

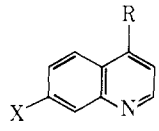
Thus, regression analyses involving these two subgroups with fixed N separations indicate that, consistent with O'Brien and Hahn's model, antimalarial activity does depend on the separation of the nitrogens in the 4-diamino side chain. When this effect is taken into consideration, the charge on the terminal N can be correlated with antimalarial activity if it is considered along with the Hansch parameter.

Analyses Involving Series I and III Combined.—The qualified success achieved with series I and III, individually, and the complete failure to obtain correlation in series II suggest that the poor results obtained for the three series combined might be due largely to the influence of series II along with the apparent steric effects implicated for series I and III. Accordingly, analyses were carried out on series I and III combined.

First, ignoring the possible steric effects, all 21 molecules of the combined series were included in the regression analyses. The combination of parameters considered and the results obtained are presented in Table XII. Comparisons of these results with those of Table IV reveals that although the significance and explained variance associated with π^2 are increased somewhat, no appreciable improvement was achieved by eliminating series II.

TABLE XII

REGRESSION ANALYSES RESULTS FOR SERIES I AND III COMBINED



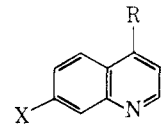
$f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}{}^+)$	R^2	Significance level, %	eV
$a\pi + b$	0.12	85	0.07
$a\pi^2 + b$	0.25	97.5	0.21
$a\pi^2 + b\pi + c$	0.34	97.5	0.27
$aQ_X + b$	0.00	<25	−0.05
$aQ_{N1} + b$	0.04	55	−0.01
$aQ_{N2}{}^+ + b$	0.10	80	0.06
$aQ_{N1} + bQ_{N2}{}^+ + c$	0.11	60	0.01
$a\pi^2 + b\pi + cQ_X + dQ_{N1} + eQ_{N2}{}^+ + f$	0.43	88	0.24

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}{}^+)$.

To make allowance for the possible steric hinderance to intercalation and dependence on diamino N separation, the analyses were repeated using only those molecules from series I with X either H or a halogen (5 molecules) and those from series III with 4 C separating the diamino nitrogens (8 molecules, chloroquine is common to both series). The combinations of parameters considered and results obtained are presented in Table XIII. Comparison of these results with those in Table XII reveal that correlation with Q_X is improved remarkably while those parameters which produce correlation in series III, (π^2, π, Q_{N1}, $Q_{N2}{}^+$) have become even less significant. Within the limitations of the assumptions and approximations inherent in these calculations, one can only conclude that the parameter Q_X dominates those associated with the diamino side chain. A rationale in terms of the model of O'Brien and Hahn might then be that the stability of the drug–DNA complex is determined primarily by the substituent at position 7 and, that varying this substituent varies the orientation of the drug relative to the DNA bases and backbones. This would then alter the manner in which the diamino side chain is able to interact with the backbone group.

TABLE XIII

REGRESSION ANALYSES RESULTS FOR SERIES I AND III COMBINED. MOLECULES WITH HYDROGEN OR HALOGEN ATTACHED AT POSITION 7 WITH 4-DIAMINO NITROGENS SEPARATED BY 4 C



$f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}{}^+)$	R^2	Significance level, %	eV
$a\pi + b$	0.05	50	−0.04
$a\pi^2 + b$	0.05	50	−0.03
$a\pi^2 + b\pi + c$	0.06	25	−0.13
$aQ_X + b$	0.51	99	0.47
$aQ_{N1} + b$	0.00	<25	−0.09
$aQ_{N2}{}^+ + b$	0.03	40	−0.06
$aQ_{N1} + bQ_{N2}{}^+ + c$	0.03	<25	−0.16
$a\pi^2 + b\pi + cQ_{N1} + dQ_{N2}{}^+ + e$	0.07	<25	−0.39
$a\pi^2 + b\pi + cQ_X + dQ_{N1} + eQ_{N2}{}^+ + f$	0.63	85	0.37

[a] Log (0.1/METD) = $f(\pi^2, \pi, Q_X, Q_{N1}, Q_{N2}{}^+)$.

Discussion

One of the principal objectives of this study was to evaluate quantitatively what appear qualitatively to be relationships between the structure of chloroquine analogs and their corresponding antimalarial activities. To this end, a free energy related structural activity equation (eq 11) was designed to incorporate those structural characteristics which have been suggested to be important. It has been found that when single structural variations are isolated, substantial correlation can be obtained under further restricted conditions. No appreciable correlation was found with any combination of the structural parameters (π^2, π, Q_X, Q_{N1}, $Q_{N2}{}^+$), however, when more than one type of structural variation was present in the series of analogs under consideration.

The results of the analyses for the individual series are, by and large, consistent with the model proposed by O'Brien and Hahn. Particularly noteworthy are the dramatically improved values for explained variance obtained with series I and with series III when allowances were made for steric requirements inherent in the model. The total lack of correlation found here for series II is in agreement with the comments of O'Brien and Hahn but no convincing rationale for this behavior has been offered.

The results of the calculation suggest two possible refinements of the O'Brien and Hahn model. With regard to the role of the diamino side chain, the apparent cooperative effect observed for the parameters $Q_{N2}{}^+$ and π might be interpreted as an indication that the size of the alkyl groups on the terminal nitrogen significantly moderates the ability of that nitrogen to participate in

electrostatic binding to a DNA phosphate group. Loss of correlation found with the parameters associated with the diamino side chain in series III when series I and III were combined suggests that perhaps different substituents at position 7 dictate different drug–DNA orientations which therein alter the manner in which the 4-diamino side chain is able to interact with the DNA backbone.

Antimalarials. 7-Chloro-4-(substituted amino)quinolines

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Received October 23, 1970

Forty-one derivatives of 7-chloro-4-substituted quinolines were made and tested for their antimalarial activity against *Plasmodium berghei* in mice. Twenty-seven showed activity.

We have previously prepared[1] many substituted 7-chloroquinolines incorporating several novel features in the side-chain amines attached at position 4. The derivatives described in the present paper do not fall into a single class as did the compounds previously reported, but cover a wide variation of substitutional features in the side chain (Table I).

Compounds **24**, **26–28**, and **37** have been described by Carmack, *et al.*[2] The alcohol **24** was used as a key intermediate. We have also used this alcohol for the preparation of **29–36**, using their procedure with slight modification. Similarly, we have used the alcohol **9** as starting material for many derivatives, but in this case, we were able to isolate the comparatively more stable bromo derivative **11** and use it for reaction with various amines.

The acetates **10** and **25** were obtained in our attempted oxidation of the alcohols **9** and **24** with DMSO in Ac_2O according to the procedure described by Burdon and Moffat.[3] No oxidation took place even when the variation described by Albright and Goldman[4,5] was used.

Some of the amines required for this work were made by using literature procedures, *e.g.*, benzylcyclopropylamine,[6] 3,4,5-trimethoxybenzylmethylamine,[7] and indanylpropargylamine.[8] The reduction of the benzylidene intermediate was carried out with $NaBH_4$ instead of using catalysts as prescribed in the literature procedures. 3,4,5-Trimethoxybenzylcyclopropylamine was prepared in the same way. Preparation of the indanylpropargylamine by the literature procedure, *i.e.*, by the alkylation of indanylamine with propargyl bromide, gave a very poor yield of the monopropargylamine. However, this difficulty was solved by formylating the indanylamine and then alkylating it with propargyl bromide. The resulting formyl derivative was hydrolyzed by treating with 3 *N* HCl.

Biological Tests.—The compounds were tested for their antimalarial activity against *Plasmodium berghei* in mice, by Dr. L. Rane, *et al.*,[9] by a procedure reported by them. The active compounds and their activity figures are listed in Table II. All others were found to be inactive.

Experimental Section

Preparation of 1, 2, 6, 7, 39, and 40.—A mixt of a 1 *M* portion of 4,7-dichloroquinoline and 2 *M* portions of the amine (or the amine·HCl as in **6**, **7**, and **39**, in which excess of anhyd K_2CO_3 was also added) in ethoxyethanol was refluxed for 24 hr. The mixt was cooled and filtered (to remove K_2CO_3 if any), and ethoxyethanol was removed under reduced pressure. The residue was mixed with H_2O, basified with NaOH soln, and extd with Et_2O or CH_2Cl_2, and the ext was dried (K_2CO_3) and concd. The residue was either distd or crystd.

For **40**, a 10 *M* excess of piperazine was used which was later distd off after the removal of ethoxyethanol, and the residue was worked up as usual.

Preparation of 5, 9, and 24.—A modification of procedure A of Surrey and Hammer[10] was used. After the mixt was heated at 150–165° for 6–8 hr, the excess amine was removed by distn under reduced pressure, and the residue cooled and triturated with dil NaOH soln. The product, which generally solidified at this stage, was then purified by crystn.

Preparation of 3, 4, 38, and 41.—A modification of procedure B of Surrey and Hammer[10] was used. After the phenol soln of 4,7-dichloroquinoline and 1–2 *M* portions of the amine was heated at 150–165° for 4–8 hr, the mixt was cooled and poured into 20% soln of NaOH. After stirring and triturating the product generally solidified, and was then purified by crystn. For **41**, a slight excess of 4,7-dichloroquinoline was used to eliminate the formation of the monoproduct.

7-Chloro-4-(3-bromo-1-methylpropylamino)quinoline (11).—To a soln of 44 ml of concd H_2SO_4 in 125 ml of 48% HBr, maintained at 0°, was slowly added 25.0 g (0.1 mole) of **9**. The mixt was then heated to boiling and maintained at boiling till (*ca.* 10 min) a turbidity was formed. After further boiling for 3 min, the mixt was cooled to room temp and extd with $CHCl_3$. The ext was washed with 10% NaOH and then with H_2O. It was dried ($MgSO_4$), filtered, and concd. The product was purified by crystn.

7-Chloro-4-[3-(*N*-phthalimido)-1-methylpropylamino]quinoline (12).—A suspension of 11 (30.0 g, 0.096 mole) and K phthalimide (22.0 g, 0.12 mole) in 500 ml of DMF was heated at 70–80° for 18 hr. The mixt was cooled, dild with 1 l. of H_2O, and extd with

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