Molecular Structural Effects Involved in the Interaction of Quinolinemethanolamines with DNA. Implications for Antimalarial Action

Michael W. Davidson, Billy G. Griggs, David W. Boykin, and W. David Wilson*

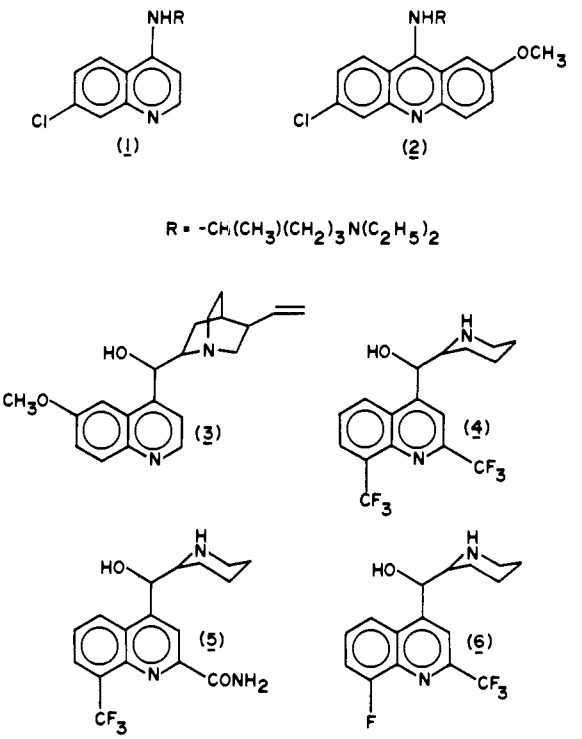
Department of Chemistry, Georgia State University, Atlanta, Georgia 30303. *Received February 3, 1977*

The interaction of mefloquine (4), two of its analogues, 5 and 6, quinacrine (2), chloroquine (1), and quinine (3) with DNA has been investigated using difference spectroscopy, spectral shifts in the presence of DNA, viscometric titrations with sonicated calf thymus DNA and Col E_1 plasmid superhelical DNA, and T_m measurements. The results from these experiments indicate that quinolines with the methanolamine side chain at position 4 cannot intercalate with DNA if they have another bulky substituent (such as trifluoromethyl) at position 2. Mefloquine (4), which has been found to be clinically effective against chloroquine-resistant *Plasmodium falciparum*, completely clears parasitemia in a single dose. This drug contains trifluoromethyl substituents at positions 2 and 8 of the quinoline nucleus and binds to DNA only weakly by electrostatic attraction at low ionic strength. Similar compounds such as 5 and quinine (3) without bulky substituents at position 2 can intercalate with DNA, but this interaction is not correlated in any apparent manner with antimalarial activity. Even intercalating quinolinemethanolamines bind weakly to DNA relative to compounds such as quinacrine (2), ethidium, and daunorubicin which are thought to exert their medicinal effects through in vivo intercalation with DNA. These results, taken collectively, strongly suggest that interaction with DNA is not involved in the antimalarial action of the quinolinemethanolamines analyzed in these experiments.

Deoxyribonucleic acid has been implicated as the in vivo receptor for antineoplastic drugs such as the anthracyclines,[1-3] actinomycin,[4,5] and coralyne,[6,7] antitrypanosomal drugs such as the phenanthridines,[8,9] and antimalarial drugs of quite varied structure.[10-13] A considerable amount of the early work on the binding of antimalarials to DNA was done by Hahn and co-workers.[12] They presented evidence that compounds such as chloroquine (1), quinacrine (2), and quinine (3) can intercalate with DNA by the classical model of Lerman[11] and suggested that intercalation was a part of their mechanism of antimalarial action. The evidence for this model, its simplicity, and the lack of a definite bioreceptor for alternative models[14,15] have helped it gain widespread acceptance. Synthetic chemists have used the model to design new antimalarial drugs,[16] and the assumption has been made that other arylmethanolamines and related compounds of similar structure involve DNA binding in their mechanism of antimalarial action.[17]

Mefloquine (4), originally prepared by Lutz and coworkers,[18] is the most successful compound to arise from the synthetic efforts to find a drug active against chloroquine-resistant strains of malaria. It has been reported from recent clinical studies with this compound that in a single oral dose it is capable of completely eliminating chloroquine-resistant *Plasmodium falciparum* from infected individuals.[19] Its structure contains apparent similarities to quinine (3) and other antimalarial drugs which give viscosity increases typical of intercalating compounds.[12,13] It has been postulated on these grounds that mefloquine (4) intercalates with DNA and that this is involved in its antimalarial action.[17]

As part of our study of antimalarials,[13,20] we have investigated the binding of mefloquine to DNA[21] and surprisingly found this interaction to be quite weak. The two bulky trifluoromethyl substituents at positions 2 and 8 along with the side chain at position 4 greatly inhibit binding relative to other arylmethanolamines.[13] In the work reported here we have used the sensitive techniques of viscometric titration utilizing sonicated and closed circular superhelical DNA along with difference spectroscopy, spectral shifts, DNA denaturation, and inhibition of *Escherichia coli* RNA polymerase to probe the interaction of quinine (3), mefloquine (4), and two trifluoromethyl-containing analogues, 5 and 6, with DNA. The results confirm our previous suggestion[21] that antimalarial activity is not correlated with DNA binding for quinolinemethanols related to mefloquine (4). We have also found that the interaction of quinolinemethanols with DNA is strongly influenced by the position and type of substituent on the quinoline nucleus.



Experimental Section

Antimalarial Agents. With the exception of quinine (3), the quinolinemethanolamines employed in this investigation were supplied by Dr. E. A. Steck of the Walter Reed Army Institute of Research. After drying to constant weight, the compounds were characterized by TLC, melting point, and spectrophotometry. All compounds yielded a single spot with TLC and the melting points and spectral analysis agreed with published values.[18] Quinine monohydrochloride dihydrate was obtained from Aldrich Chemical Co. (Lot No. 042217). Quinacrine dihydrochloride, chloroquine diphosphate, and putrescine dihydrochloride were obtained from Sigma (Lots 94C-0080, 39B-1070, and 35B-2400, respectively). Commercially prepared compounds were analyzed as described above. Daunorubicin was generously provided by Dr. Harry B. Wood, Jr., of the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Concentrated drug stock solutions ($1-3 \times 10^{-3}$ M) were prepared in standard buffer (3.8×10^{-3} M NaH_2PO_4, 5×10^{-4} M EDTA, adjusted to pH 7.0 with NaOH, ionic strength 0.0095) and found to be stable for a period

of 6 months when stored in the dark at 4 °C.

Calf Thymus DNA. DNA obtained from Miles Laboratories, Inc. (Lot No. 36-155), was dissolved in standard buffer to a final concentration of 5–7 × 10^{-3} M and dialyzed against 1000 vol of standard buffer. This DNA was used in thermal denaturation studies. Calf thymus DNA obtained from Worthington Biochemicals (Lot No. 35M614) was dissolved to a concentration of 3.5 mg/mL in standard buffer, made 2.0 M in NaCl by the addition of solid NaCl, and then subjected to sonication in 30-s intervals with a Blackstone Ultrasonics SS-2 sonifier tuned for maximum output. Temperature was maintained between 4 and 8 °C by chilling the DNA solution in an ice–salt bath during the sonication procedure. Total sonication time was 1 h after which the DNA solution was passed through a prewashed membrane filter (0.45 μ from Millipore) to remove particulate contamination, and the DNA was then precipitated by addition of an equal volume of absolute ethanol. DNA was redissolved and adjusted to a concentration of approximately 2 × 10^{-2} M in standard buffer and lyophilized for storage. When ready for use, lyophilized DNA was redissolved in the appropriate buffer and dialyzed against 1000 vol of the same buffer. DNA prepared in this manner displays an A_{260}/A_{280} ratio between 1.89 and 1.93, an A_{260}/A_{230} ratio between 2.25 and 2.4, and a T_m in standard buffer of 62 °C. No change in total hyperchromicity at 260 nm was detected between sonicated and untreated Worthington DNA (the value is usually between 29 and 30%). The viscosity-average molecular weight for sonicated DNA was evaluated in a capillary viscometer and found to be 5.2 × 10^5 daltons from $[\eta]$ = 3.66 dL/g utilizing the equation of Eigner and Doty.[22] All DNA concentrations were determined using an extinction coefficient of 6600 M^{-1} cm^{-1} and are expressed as nucleotide equivalents per liter.

Colicinogenic Factor E_1 DNA. A strain of *E. coli* harboring the colicinogenic factor E_1 (Col E_1) plasmid, JC 411 *thyA* (Col E_1), was obtained from Dr. Daniel Vapnek of the Program in Genetics, Department of Microbiology, University of Georgia. Samples of JC 411 *thyA* (Col E_1) were removed from a 4-mL stab agar bottle and streaked on petri nutrient agar plates containing 2 μg/mL of thymine. After incubation at 25 °C for 24 h visible colonies appeared which could be easily isolated. A single colony was carefully picked with a sterile loop and transferred to 50 mL of K medium;[23] the culture was placed in a New Brunswick gyratory controlled environment incubator/shaker (Model No. G-25) and rotated at 200 rpm and 37 °C for 12 h. This culture was then used as an inoculum for a 10-L culture which was grown with vigorous aeration at 37 °C. When the bacterial population reached 3–5 × 10^8 cells/mL, solid chloramphenicol (Sigma Chemical Co. Lot No. 73C-1250) was added to a final concentration of 200 μg/mL and incubation continued for an additional 15–20 h to allow maximum amplification of plasmid DNA.[24] At the end of this time period, the cells were harvested by centrifugation at 7000 rpm (10 100g) for 20 min in a Sorvall GS-3 rotor. Bacterial pellets were resuspended in 15 mL of sucrose–Tris buffer (25% sucrose; 0.05 M Tris, pH 8.0) per liter of original culture and stored at 4 °C for 10 min. The suspension was then treated with lysozyme (Worthington Lot No. 34P797) by addition of a concentrated stock solution (10 mg/mL in 0.05 M Tris, pH 8.0) to a final concentration of 2 mg/mL and again stored at 4 °C for 10 min.[25] The thick cellular suspension was then adjusted to 6 × 10^{-2} M in EDTA by addition of 1 mL of 0.25 M EDTA, pH 8.0, per 3 mL of bacterial suspension and the resulting mixture was swirled and incubated for 30 min at 4 °C. Lysis of the bacterial spheroplasts was achieved by addition of 5 M NaCl to a final concentration of 1 M (0.25 mL/mL of suspension), followed by gentle stirring and dropwise addition of 10% sodium dodecyl sulfate (SDS) until a concentration of 1% SDS was attained. Lysis occurs rapidly but in some instances it may be necessary to incubate at 37 °C with shaking for 30–45 min to completely clarify the solution before allowing the viscous lysate to stand overnight at 4 °C. The lysate was then transferred to 50-mL polyallomer tubes and centrifuged at 17 000 rpm (34 800g) for 90 min in a Sorvall SS-34 rotor. After centrifugation, the supernatant solution was carefully removed and recentrifuged to remove any additional cell debris, precipitated SDS, or contaminating high-molecular-weight chromosomal DNA. Next, the supernatant solution was dialyzed twice against 6 L of SSC–EDTA (0.15 M NaCl, 0.015 M Na citrate, 0.01 M EDTA, pH 7.0) and CsCl (Gibco Lot No. RO62556) was slowly added with stirring to a final concentration of 0.775 g/mL. After the CsCl had completely dissolved, a 10 mg/mL solution of ethidium bromide (Sigma Chemical Co. Lot No. 121C-0330) in distilled water was added dropwise to give a concentration of 300 μg/mL. The solution was then transferred to polyallomer tubes and centrifuged at 34 000 rpm (92 500g) and 17 °C for 96 h in a Spinco 35 rotor. After centrifugation, two bands were distinctly visible in the center portion of the tubes, the upper band consisting of linear and nicked circular DNA and the bottom band being composed of closed circular superhelical DNA.[26] Visualization of the DNA bands was enhanced by illuminating the tubes with a long-wavelength (bandpass center at 366 nm) ultraviolet lamp (Ultraviolet Products, Inc.) and the bottom band was carefully collected with a syringe. Ethidium was removed by repeated extraction with CsCl-saturated 2-propanol[27] and CsCl was removed by dialysis against 18 L of SSC–EDTA. DNA was then precipitated by addition of an equal volume of ethanol and the DNA precipitate collected by centrifugation at 10 000 rpm (12 100g) in a Sorvall SS-34 rotor. DNA was redissolved in SSC–EDTA (50 mL) and mixed with CsCl and ethidium bromide as described above to bring the total volume to 90 mL. The resulting solution was transferred to cellulose nitrate tubes and centrifuged at 35 000 rpm (16 500g) and 17 °C for 72 h in a Spinco SW-40 Ti rotor. The second isopycnic density gradient treatment was necessary to remove large amounts of RNA which contaminated the lower DNA bands in the first centrifugation. Closed circular superhelical DNA was collected and the ethidium removed as described above before subjecting the DNA to dialysis against 1000 vol of standard buffer. Purified Col E_1 DNA has an A_{260}/A_{280} ratio between 1.89 and 1.95 and an A_{260}/A_{230} ratio from 2.20 to 2.45. Fluorescence analysis, utilizing a Perkin-Elmer MPF-44A spectrofluorometer, indicated that the residual ethidium in this DNA is less than 0.01% on a molar basis. This is insignificant for any of our experiments.

Spectral Shifts. The electronic absorption spectra of quinolinemethanols **3–6** were recorded in standard buffer utilizing 0.2-cm lightpath quartz cuvettes (Pyrocell) and a Cary 17D spectrophotometer. After each spectrum was recorded, the sample cuvette was filled with a solution of the appropriate quinolinemethanol at the same concentration but with a fivefold excess of sonicated calf thymus DNA. The reference cuvette for this second spectrum was filled with a solution of DNA alone at the same concentration as in the sample cuvette, and the spectrum was recorded.

Difference Spectra. All the difference spectral experiments were conducted on either a Cary 17D or Beckman Acta V spectrophotometer. Cylindrical tandem cells (10 by 10 mm) from Pyrocell were filled with DNA solution (2.3 × 10^{-3} M) and drug solution (3.5 × 10^{-4} M) in separate compartments and a baseline was determined. Equal volumes of DNA and drug solutions were then mixed and analyzed against a reference of the separated solutions. Because of the difficulty in pipetting these concentrated drug and DNA solutions, the equal volumes were obtained by filling two calibrated 5-mL volumetric flasks to the mark and throughly mixing these two solutions. No significant changes in drug extinction were obtained diluting over this concentration range.

Viscosity Measurements. Relative viscosities were recorded in a thermostated water bath at 25 °C with a Cannon-Ubbelohde size 75 semimicro dilution viscometer. In titrations utilizing sonicated calf thymus DNA, the viscometer reservoir was filled with a total volume of 1.0–3.0 mL of DNA solution and small aliquots of concentrated drug stock solution were carefully added with the aid of a microliter syringe (Hamilton) affixed to a long section of glass tubing to allow additions to be made directly into the viscometer reservoir. The drug stock solution contained DNA at the same concentration as in the viscometer to circumvent dilution effects. In the case of Col E_1 DNA, the DNA solution volume in the viscometer ranged between 0.65 and 1.0 mL. Successive aliquots of concentrated drug stock solution were added as before except the drug stock was free of DNA. The total volume change never exceeded 3% and dilution of DNA during the course of the titration was corrected in reduced specific viscosity calculations.

Thermal Denaturation Profiles. Denaturation experiments were conducted in a Beckman Acta V spectrophotometer equipped

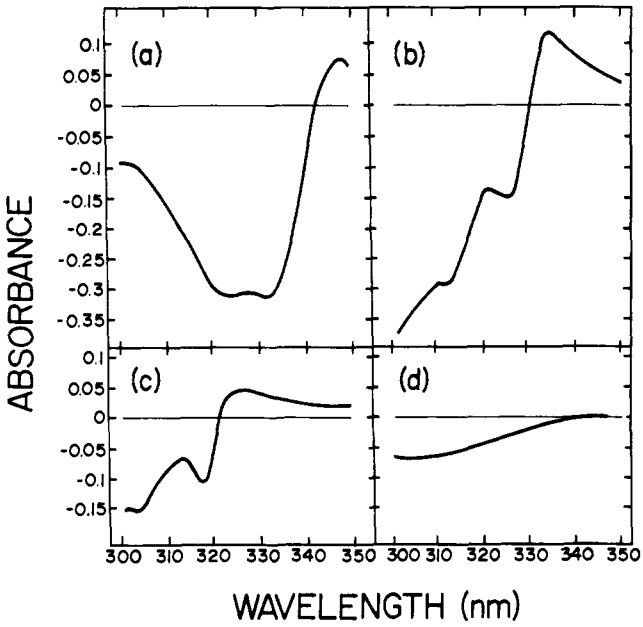


Figure 1. Difference spectra of quinolinemethanols 3–6 in the presence of sonicated calf thymus DNA: (a) quinine (3); (b) compound 5; (c) mefloquine (4); (d) compound 6. All solutions were in standard buffer.

with a water-jacketed cell block. Temperature was controlled with a Haake Model FE circulating water bath containing 50% v/v ethylene glycol. Temperature was monitored through a thermister housed in the cell block and coupled to a Beckman temperature module accessory. Absorbances were determined as a function of temperature in 1.0-cm lightpath quartz cuvettes (Pyrocell) containing tight-fitting Teflon stoppers. The sample cuvette stopper possessed a 0.05-cm aperature through the center which served to relieve pressure during high-temperature experiments, to condense vapor, and to prevent evaporation.

Inhibition of *E. coli* RNA Polymerase. The procedure for these experiments has been previously reported.[1] Drugs were added to DNA at a molar ratio of 1.0 in an attempt to detect any weak inhibition of RNA polymerization.

Miscellaneous. Measurements of pH were conducted with a Fisher Accumet (Model No. 320) expanded scale pH meter equipped with a micro-combination electrode (No. 13-639-93). Buffer salts and reagents not described above were of the highest purity commercially available. Union Carbide dialysis tubing was pretreated by boiling in two changes of 0.01 M $NaHCO_3$, followed by five changes of deionized water. Dialysis tubing and concentrated DNA stock solutions were stored at 4 °C with a few drops of $CHCl_3$. Water was glass redistilled from acid permanganate.

Results

Spectral Changes. The difference spectral technique is quite sensitive to slight changes in the chromophoric environment and, therefore, is an excellent technique for analysis of weak interactions. Difference spectra of drugs 3–6 caused by DNA under equivalent conditions in relatively concentrated solution are shown in Figure 1. As can be seen, quinine (3) and 5 have large changes in their spectra under these conditions while mefloquine (4) and 6 have much smaller changes. Spectral shifts for these compounds upon adding DNA are shown in Figure 2. Although not as sensitive as difference spectra, these results agree with those in Figure 1 and indicate a much stronger interaction with DNA for quinine (3) and 5 relative to mefloquine (4) and 6.

Viscometric Titrations. Compounds which intercalate with DNA by the Lerman model[11] should cause a dramatic increase in viscosity of low-molecular-weight (sonicated) DNA.[28] Cations which bind by other mechanisms such as simple electrostatic interaction typically cause slight decreases in DNA viscosity due to lowering of phosphate electrostatic repulsion. The results of viscometric titrations of sonicated calf thymus DNA by 1–6 are shown in Figure 3. Quinine (3) and 5 elicit a modest viscosity increase similar to that displayed by quinacrine (2) and chloroquine (1) but reduced in magnitude. In contrast, mefloquine (4) causes slight decreases in DNA viscosity suggesting a weak electrostatic interaction with the deoxyribose–phosphate backbone. Compound 6 exhibits neither an increase nor a decrease in DNA viscosity but the results are obviously closer to mefloquine (4) than to quinine (3) and 5. Titration of covalently closed circular superhelical DNA by an intercalating molecule first produces an increase in DNA viscosity as the superhelical density is decreased upon unwinding the double helix. A maximum in the titration curve is reached corresponding to a point where all superhelical turns are removed, and the viscosity again decreases as reverse turns are formed by the addition of more drug.[29] In Figure 4 viscometric titrations for the

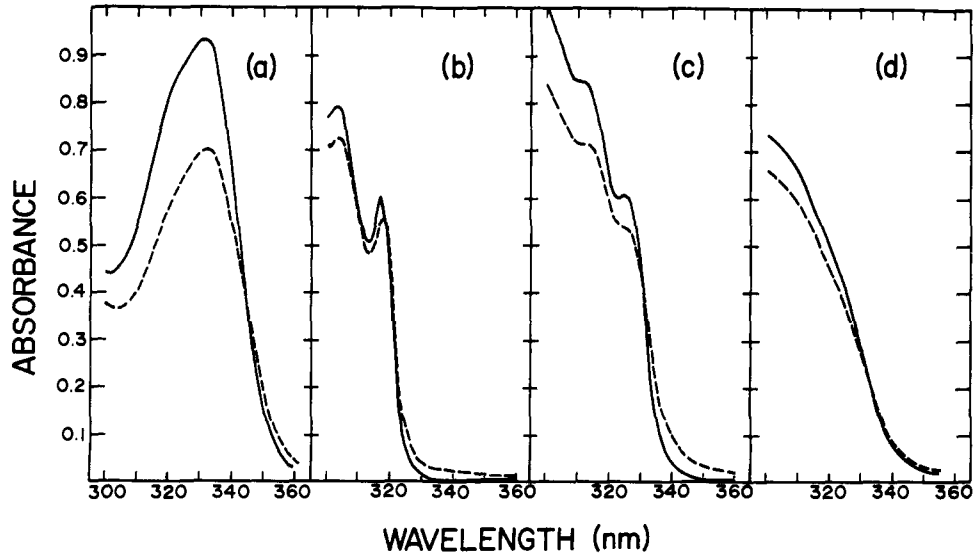


Figure 2. Effect of sonicated calf thymus DNA on the near-ultraviolet absorption spectra of quinolinemethanols 3–6: (a) quinine (3); (b) mefloquine (4); (c) compound 5; and (d) compound 6. Solid lines indicate spectra of the quinolinemethanols alone at a concentration of 10^{-3} M. Dashed lines represent spectra of quinolinemethanols (10^{-3} M) in the presence of 5×10^{-4} M DNA. All solutions were in standard buffer.

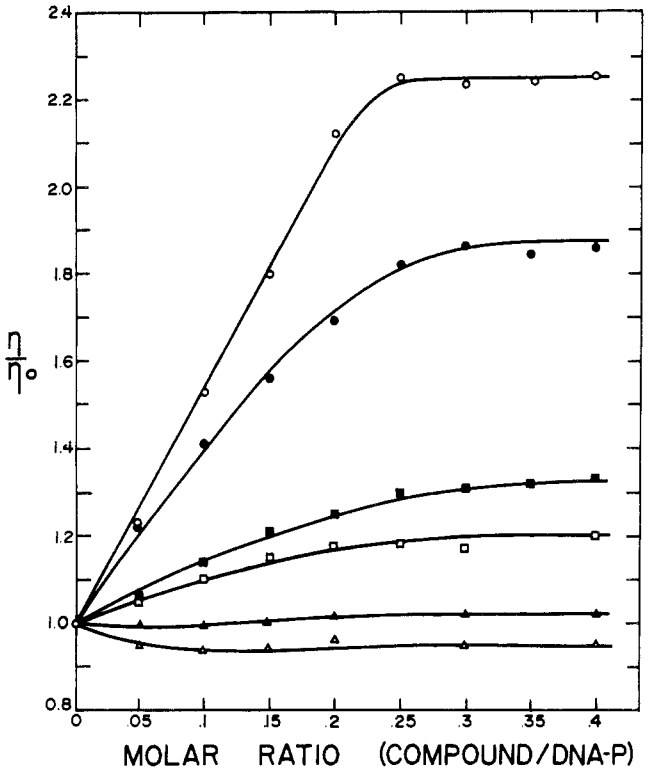


Figure 3. Effect of compounds 1–6 on the viscosity of sonicated calf thymus DNA. The reduced specific viscosity ratio (η/η_0) of DNA alone (η_0) to DNA–drug complex (η) is plotted as a function of the molar ratio of compound added per DNA nucleotide equivalent. DNA solutions were prepared in standard buffer at a concentration of 2.0×10^{-4} M. (O-O) quinacrine (2); (●-●) chloroquine (1); (□-□) quinine (3); (■-■) compound 5; (△-△) mefloquine (4); (▲-▲) compound 6.

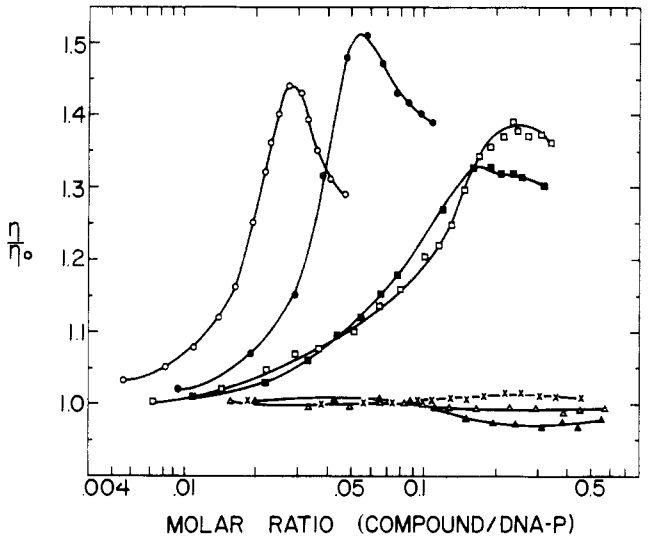


Figure 4. Viscometric titrations illustrating the interaction of compounds 1–6 and putrescine with closed circular superhelical Col E_1 DNA. The reduced specific viscosity ratio, discussed in Figure 3, is plotted as a function of drug added per Col E_1 DNA nucleotide equivalent on a logarithmic scale. DNA concentration in all cases was 2.59×10^{-4} M. (O-O) quinacrine (2); (●-●) chloroquine (1); (□-□) quinine (3); (■-■) compound 5; (△-△) mefloquine (4); (▲-▲) compound 6; (×-×) putrescine. Putrescine was included as a control to demonstrate the effect of aliphatic amines on the superhelical density of Col E_1 DNA.

intercalating antimalarial drug quinacrine (2), the weaker binding intercalating antimalarial drug chloroquine (1), and the nonintercalating diamine putrescine are shown along with results for 3–6 upon interaction with Col E_1 superhelical DNA. Compound 5 and quinine (3) act as very weak binding intercalating agents while mefloquine (4) and 6 act in a manner similar to putrescine.

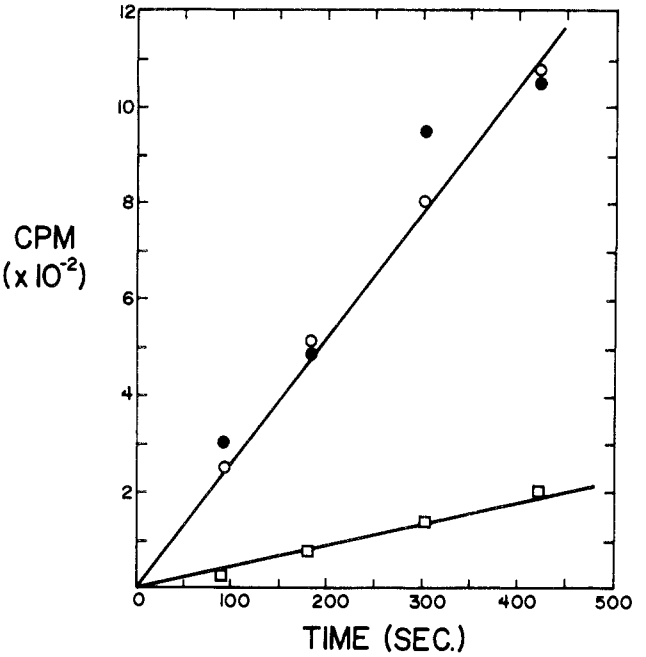


Figure 5. Effect of mefloquine (4) and daunorubicin on transcription of RNA directed by *E. coli* RNA polymerase. (●-●) mefloquine (4); (□-□) daunorubicin; (O-O) DNA control.

Inhibition of RNA Polymerase. To determine whether any undetected interaction of mefloquine (4) with DNA could account for its high antimalarial activity, we analyzed the inhibition by this compound of RNA transcription by *E. coli* RNA polymerase in a standard assay system.[1,30] As can be seen in Figure 5, mefloquine (4) has no effect on RNA transcription while daunorubicin, used as a control, greatly decreases the amount of RNA formed. As with the physical studies, this suggests that complexation with DNA is not involved in the antimalarial action of mefloquine (4).

Thermal Denaturation of DNA. Because of the very weak binding of 3–6 to DNA, attempts to determine accurate and reproducible binding constants by several techniques were unsuccessful. Typically the amount of compound bound was not significantly larger than systematic errors in the various methods available. We have, however, conducted T_m studies which are illustrated in Figure 6. The intercalating drug quinacrine (2) produces a very large increase in DNA T_m as does chloroquine (1) at a ratio of one drug molecule per ten DNA nucleotides. Quinine (3) and 5 exhibit marginal T_m increases even at these low ionic strengths (0.0095) and at a tenfold higher ratio of compound to DNA nucleotide (one drug molecule per nucleotide). The thermal denaturation profile of DNA in the presence of mefloquine (4) and 6 remains virtually unaffected. The slight increase displayed by mefloquine (4) is hardly more than would be expected for adding any organic cation to DNA at low ionic strength. Even the interaction of quinine with DNA is quite weak compared to other drugs whose mode of action has been postulated to involve interaction with DNA.[1,4,9,13]

Discussion

The results of this work clearly indicate that the role of DNA binding for all quinolinemethanols must now be reevaluated. Quinine (3), for example, binds to DNA much more weakly than other antimalarials such as quinacrine (2) and naphthothiopheneethanolamines[13,20] and other

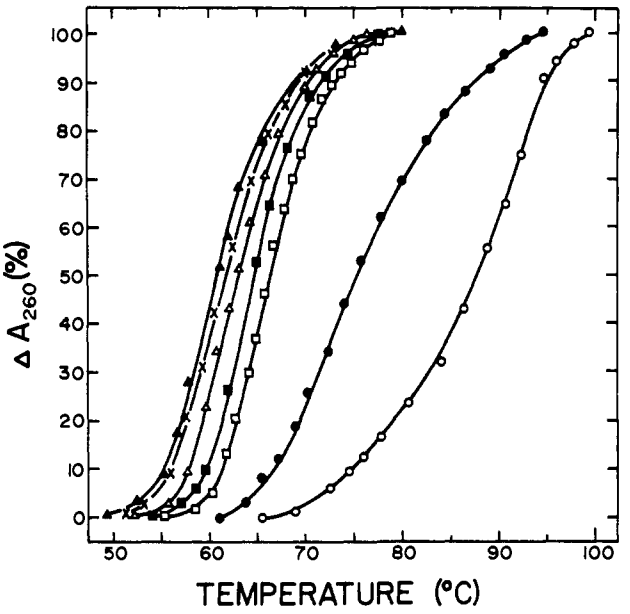


Figure 6. Thermal denaturation profiles of calf thymus DNA in the presence of 1–6. The percent change in absorbance at 260 nm is plotted as a function of temperature. Quinolinemethanols 3–6 were at a concentration of 10^{-4} M, quinacrine (2) and chloroquine (1) were at a concentration of 10^{-5} M, and the DNA concentration in all cases was 10^{-4} M. Solutions were made in standard buffer. (O-O) quinacrine (2), T_m = 88.0 °C; (●-●) chloroquine (1), T_m = 75.0 °C; (□-□) quinine (3), T_m = 66.3 °C; (■-■) 5, T_m = 64.9 °C; (Δ-Δ) mefloquine (4), T_m = 63.0 °C; (×-×) DNA control, T_m = 62.0 °C; (▲-▲) 6, T_m = 61.5 °C.

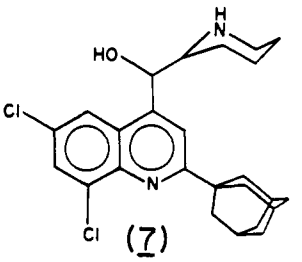
Table I. Antimalarial Results[a]

Compd no.	Dosage, mg/kg				
	40	80	160	320	640
4[b]	3C	5C	5C	5C	5C
5	0.3	0.3	0.5	0.7	7.9
6[c]	0.3	4.1	5.1	7.1	10.5

[a] Test data, communicated by E. A. Steck of the Walter Reed Army Institute of Research, against *P. berghei* in mice. Increase in mean survival time (ΔMST) of the controlled group is reported. A compound is active if the increase in MST exceeds 6.1 days and curative (C) if one or more of the five tested mice live 60 days postinfection. [b] See ref 18. [c] See ref 37.

clinically useful drugs such as daunorubicin,[1,3] ethidium bromide,[8] and actinomycin D,[4] whose medicinal action is thought to involve DNA binding. The antimalarial activities of 4–6 are given in Table I. Compound 5 clearly can intercalate with DNA and binds more strongly than mefloquine (4) but is devoid of antimalarial activity while mefloquine (4) is highly active and has displayed effective results in clinical trials.[19]

Model building studies with CPK space-filling molecular models coupled to the experimental results obtained with 4 and 6 indicate that these compounds and other active antimalarials[31] such as 7 cannot intercalate and, at best, bind only weakly to the DNA phosphate groups. Such compounds must have some in vivo receptor other than DNA. Alternative mechanisms for antimalarial action of these compounds have been recently reviewed by Peters.[15,32] Chloroquine (1) and related 4-aminoquinolines cause clumping of the malaria pigment which forms as the parasite digests host cell hemoglobin. The mechanism of action proposed by Peters, Warhurst, and co-workers[32,33] assumes that this clumping disrupts the production of amino acids needed by the parasite leading to its death. Interaction with DNA is assumed to be a secondary effect,



(7)

occurring only as the parasite dies, and to have no antimalarial significance. Arylmethanolamines including mefloquine (4), which are active antimalarials, inhibit this clumping[33] but exactly how they exert their antimalarial action is not clear.

Considerable work concerning the effects of drugs on *Plasmodium berghei* in cell culture has been conducted by Van Dyke and co-workers.[14,34] They find that acridine and phenanthridine drugs which bind strongly to DNA [such as quinacrine (2) illustrated in our experiments] inhibit polymerization of nucleic acids in the parasite. Compounds binding more weakly to DNA such as chloroquine (1) and quinine (3) do not affect nucleic acid polymerization at normal drug dosages. They conclude that there must be some mechanism of antimalarial action other than intercalation with DNA for such compounds.[14] Their results strongly support our conclusions with mefloquine (4). This leaves the unfortunate result that the mechanism of action of many quinolinemethanolamines, including perhaps the original antimalarial, quinine (3), cannot be specified at present.

A finding from this work important for understanding small molecule–DNA interactions is that the ability of quinolinemethanolamines to intercalate with DNA is strongly dependent on the location and type of substituent placed on an aromatic ring. We refer to a bulky substituent as one which extends significantly beyond the 3.4-Å thickness of a typical fused aromatic ring system and which can interfere with stacking in an intercalated complex. For 3–6 this includes only the side chain and trifluoromethyl substituents. CPK space-filling models indicate that the methoxy group of quinine (3) and the carboxamide group of 5 can be rotated into the plane of the quinoline ring system. All compounds contain a bulky group on position 4 (the side chain). Two compounds, 4 and 6, do not intercalate and both have an additional bulky group at position 2. Two compounds intercalate [quinine (3) and 5] and neither contains a bulky substituent at position 2, although 5 does have an additional bulky substituent at position 8. A study of possible interactions of all four compounds with DNA using CPK space-filling molecular models indicates that the trifluoromethyl substituent of 5 can project into one groove of the DNA double helix leaving the side chain in the opposite groove. Such a complex allows stacking of the quinoline ring system and the carboxamide substituent with the DNA base pairs. Similar stacking is obtained with quinine (3) since it contains only one bulky substituent, the side chain.

The proximity to the side chain of the additional bulky substituent at position 2 in 4 and 6, however, prevents the two substituents from lying in opposite grooves of the double helix. This would require that, for intercalation with DNA, one bulky substituent would have to be partially pulled between the base pairs, resulting in a severe disruption of stacking. Alternately, both bulky substituents could lie in the same groove allowing at least partial insertion of the ring system of 5 in a manner described by Gabbay and co-workers.[35,36] This, however, would lead to large decreases in sonicated DNA viscosity in contrast to

our results illustrated in Figure 5. The viscometric studies suggest that both mefloquine (4) and 6 simply interact with DNA through weak external counterion electrostatic attraction.

We have shown with naphthothiopheneethanolamines that addition of a trifluoromethyl substituent to a planar aromatic ring system can actually enhance DNA binding.[13,20] In this case, however, model building studies indicated that the side chain can lie in one groove of the DNA double helix and the trifluoromethyl substituent in the other. The enhancement of binding on introduction of a trifluoromethyl substituent in this system is presumably due to electronic factors, a point which we have under investigation. Positioning of bulky substituents on small molecules can, thus, lead either to significant increases or to decreases in DNA binding depending on their relative position with respect to other bulky substituents, the electronic characteristics of the substituent, and the structure of the DNA–drug complex.

Acknowledgment. The authors thank Dr. E. A. Steck of the Walter Reed Army Institute of Research for supplying samples of compounds 4–6 and for encouragement and helpful advice in this research. Dr. Harry B. Wood, Jr., of the National Cancer Institute generously supplied us with a daunorubicin sample. We thank Dr. Daniel Vapnek for supplying a culture of *E. coli* JC 411 *thyA* (Col E_1) and for helpful advice on its growth and isolation of plasmid DNA. This work was supported in part by grants from the Research Corporation and from the Georgia State University Arts and Sciences Research Fund.

References and Notes

(1) W. D. Wilson, D. Grier, R. Reimer, J. Bauman, J. Preston, and E. Gabbay, *J. Med. Chem.*, **19**, 381 (1976).
(2) G. Tong, W. Lee, D. Black, and D. W. Henry, *J. Med. Chem.*, **19**, 395 (1976).
(3) A. DiMarco and F. Arcamone, *Arzneim.-Forsch.*, **25**, 368 (1975).
(4) W. Mueller and D. M. Crothers, *J. Mol. Biol.*, **35**, 251 (1968).
(5) S. C. Jain and H. M. Sobell, *J. Mol. Biol.*, **68**, 1 (1972); H. M. Sobell and S. C. Jain, *ibid.*, **68**, 21 (1972).
(6) K. Y. Zee-Cheng and C. C. Cheng, *J. Pharm. Sci.*, **62**, 1572 (1973).
(7) W. D. Wilson, A. N. Gough, J. J. Doyle, and M. W. Davidson, *J. Med. Chem.*, **19**, 1263 (1976).
(8) M. J. Waring, *J. Mol. Biol.*, **13**, 269 (1965).
(9) M. J. Waring, "The Molecular Basis of Antibiotic Action", E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring, Ed., Wiley, London, 1972, pp 173–277.
(10) E. A. Steck, "Chemotherapy of Protozoan Diseases", U.S. Government Printing Office, Washington, D.C., 1972, pp 23, 77–123, 177.
(11) L. S. Lerman, *Proc. Natl. Acad. Sci. U.S.A.*, **49**, 94 (1963).
(12) F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Olenick, *Mil. Med.*, **131**, 1071 (1966).
(13) E. J. Olmstead, J. W. Panter, D. W. Boykin, and W. D. Wilson, *Biochemistry*, **14**, 521 (1975).
(14) G. Carter and K. Van Dyke, *Proc. Helminthol. Soc. Wash.*, **39**, 244 (1972).
(15) W. Peters, "Advances in Parasitology", B. Dawes, Ed., Academic Press, London, 1974, pp 69–114, and references cited therein.
(16) V. E. Marquez, J. W. Crauston, R. W. Ruldon, and J. H. Burckhalter, *J. Med. Chem.*, **17**, 856 (1974).
(17) D. W. Henry, *Natl. Med. Chem. Symp. Am. Chem. Soc., Proc.*, **13**, 41 (1972).
(18) C. J. Ohnmacht, A. R. Patel, and R. E. Lutz, *J. Med. Chem.*, **14**, 926 (1971).
(19) G. M. Trenholme, R. L. Williams, R. E. Desjardim, H. Frischer, P. E. Carson, K. H. Rieckmann, and C. J. Canfield, *Science*, **190**, 792 (1975).
(20) J. W. Panter, D. W. Boykin, and W. D. Wilson, *J. Med. Chem.*, **16**, 1366 (1973).
(21) M. W. Davidson, B. G. Griggs, D. W. Boykin, and W. D. Wilson, *Nature (London)*, **254**, 632 (1975).
(22) J. Eigner and P. Doty, *J. Mol. Biol.*, **12**, 549 (1965).
(23) D. Vapnek, N. K. Alton, C. L. Bassett, and S. R. Kushner, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3492 (1976).
(24) D. Clewell, *J. Bacteriol.*, **110**, 667 (1972).
(25) J. Sidikaro and M. Nomura, *J. Biol. Chem.*, **250**, 1123 (1975).
(26) R. Radloff, W. Bauer, and J. Vinograd, *Proc. Natl. Acad. Sci. U.S.A.*, **57**, 1514 (1967).
(27) R. Davis and D. Vapnek, *J. Bacteriol.*, **125**, 1148 (1976).
(28) G. Cohen and H. Eisenberg, *Biopolymers*, **8**, 45 (1969).
(29) B. M. J. Revet, M. Schmir, and J. Vinograd, *Nature (London), New Biol.*, **229**, 10 (1971).
(30) J. F. Preston, H. S. Stark, and J. W. Kimbrough, *Lloydia*, **38**, 153 (1975).
(31) J. Novotny, C. Collins, and F. Starks, *J. Pharm. Sci.*, **63**, 1264 (1974).
(32) W. Peters, *Postgrad. Med. J.*, **49**, 573 (1973); D. C. Warhurst, C. A. Homewood, W. Peters, and V. C. Baggaley, *Proc. Helminthol. Soc. Wash.*, **39** (special issue), 271 (1972).
(33) D. C. Warhurst and S. C. Thomas, *Biochem. Pharmacol.*, **24**, 2047 (1975).
(34) K. Van Dyke, C. Lantz, and C. Szustkiewicz, *Science*, **169**, 493 (1970); H. Lantz and K. Van Dyke, *Biochem. Pharmacol.*, **20**, 1157 (1971).
(35) E. J. Gabbay, R. De Stefano, and K. Sanford, *Biochem. Biophys. Res. Commun.*, **46**, 155 (1972).
(36) P. Adawadkar, W. D. Wilson, W. Brey, and E. J. Gabbay, *J. Am. Chem. Soc.*, **97**, 1959 (1975).
(37) A. Patel, C. Ohnmacht, D. Clifford, A. Crosby, and R. Lutz, *J. Med. Chem.*, **14**, 198 (1971).

Antimalarials. 10. Synthesis of 4-Substituted Primaquine Analogues as Candidate Antimalarials

Maurice P. LaMontagne,* Anica Markovac, and John R. Menke

Ash Stevens Inc., Detroit, Michigan 48202. *Received February 17, 1977*

Primaquine (I) has been extensively used in combination with other drugs in the radical cure of relapsing malaria as well as for prophylaxis or the interruption of transmission. This, coupled with the activity data reported for 4-methylprimaquine (II), has led to the synthesis of a series of 14 4-substituted analogues of I. In addition, three side-chain analogues of II were prepared. The compounds were tested for suppressive antimalarial activity against *Plasmodium berghei* in the Rane mouse screen and for radical curative activity against *Plasmodium cynomolgi* in the rhesus monkey. Four of the 17 compounds prepared (**1a**, **9c**, **15**, and **17**) exhibited activity in at least one of the test systems.

Several 8-quinolinediamines were synthesized during the 1940's and tested for antimalarial activity. The majority of these early compounds were 6-methoxyquinolines containing a wide variety of diamine side chains in the 8