Antimalarial Quinones for Prophylaxis Based on a Rationale of Inhibition of Electron Transfer in Plasmodium*

(coenzyme Q/malaria/antimetabolites/therapy/bioenergetics)

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ABSTRACT Knowledge of the biochemistry of Plasmodium is emerging as a new field. Previous studies showed that the parasite apparently requires electron transfer for energy, and techniques to study such energy mechanisms are available. The discovery of the existence of coenzyme Q, in Plasmodium implies an indispensable functionality for this reductant entity in the electron transfer of the parasite, as coenzyme Q, similarly functions in other forms of life.

Effective antimalarial activity in prophylaxis has been demonstrated in sporozoite-induced infections by Plasmodium gallinaceum in chicks by several representatives of 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones. The absence of toxicity in this assay even at greatly elevated dosage underscores the achievement of selectivity and safety to the host for the potential utilization of antimetabolites of coenzyme Q, as medicinals.

Seven new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones were synthesized. The structural variations of the 7-alkylmercapto group in relationship to the antimalarial activities reveal substantial differences in biological activities, which can reflect molecular specificities of enzyme sites and which are not evident from the deceptively minor structural differences in the alkylmercapto groups.

These analogs of coenzyme Q, having effective antimalarial activity are known to inhibit mammalian coenzyme Q, enzymes, and could be useful in elucidation of the basic electron transfer mechanisms of Plasmodium.

The advancing new field of the biochemistry of Plasmodium may facilitate the achievement of new antimalarial drugs. P. berghei apparently has a glycolysis mechanism. Fulton and Spooner (1) reported the utilization of oxygen, and Scheibel and Miller (2) demonstrated cytochrome oxidase in the parasite.

Homewood et al. (3) treated P. berghei with commonly used mammalian inhibitors of electron transfer and observed responses which indicated that the parasite apparently requires electron transfer for the production of energy. For example, antimycin A inhibited both oxygen uptake and clumping, and rotenone inhibited clumping. The clumping of malarial pigment after treatment, in vivo, with chloroquine is a process which requires energy (4, 5). The extent of clumping and utilization of oxygen may be used as assays in support of studies on the bioenergetics of the parasite.

The actual steps of electron transfer for P. berghei are largely unknown. Scheme I, as based on the proposal of Rothenone Antimycin A

\[
\text{NADH} \rightarrow \text{main electron transfer chain: CoQ} \rightarrow \text{Acceptor} \\
\text{Substrate} \rightarrow \text{minor electron transfer chain} \rightarrow \text{O}_2 \\
\text{Cyanide}
\]

Scheme I. Electron Transfer of P. berghei.

Homewood et al. (3) but modified to include coenzyme Q, may guide studies on the transfer steps.

The search for the presence of coenzyme Q in species of Plasmodium was successful, and mass spectral data and reversed-phase chromatographic results of Rietz et al. (6) showed the presence of coenzyme Q in P. lophurae. Vitamin K was not detected. Skelton et al. in cooperation with Schnell et al. (7) demonstrated that coenzyme Q was biosynthesized by P. knowlesi. Next, it was shown by Skelton et al. (8) that coenzyme Q was also biosynthesized by P. cynomolgi and P. berghei. Skelton et al. (9) found no evidence for the biosynthesis of vitamin K from [14C]shikimate acid by P. knowlesi, and it was concluded that coenzyme Q is the dominant form of coenzyme Q, in the electron transfer of species of Plasmodium, instead of a form of vitamin K. Coenzyme Q, could function in the mitochondria of species of Plasmodium. The presence of mitochondria in species of Plasmodium, as determined by Hepler et al. (10) and Rudzinska and Trager (11) and the dependence of the parasite upon a gas phase containing a given concentration of oxygen are known; Anfinsen et al. (12).

Drugs for the curative treatment of malaria in man are medically important, and antimalarial drugs for prophylaxis are also of value for human use. The advent of drug-resistant malaria about 1948 stimulated research toward new drugs, and new approaches were sought.

After the discovery of coenzyme Q in Plasmodium, it was evident that the synthesis of diversified analogs of coenzyme Q, and assay of their inhibition of CoQ enzymes in mammalian systems could constitute a rational selection of analogs for tests for antimalarial activity. Based on this rationale, syntheses began in 1968, and new analogs of coenzyme Q were tested in mice infected with P. berghei; the emphasis was on curative antimalarials.

Recently, the biological emphasis changed to new prophylactic activity from curative activity. It was recognized that a sporozoite-induced infection of Plasmodium gallinaceum in chicks could serve as a primary test of therapeutic and/or

Abbreviation: m.p., melting point.

* This is publication no. 168 in a series on coenzyme Q, and no. 22 in a series on antimetabolites of coenzyme Q. The preceding paper is ref. 20.

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prophylactic activity, and as a confirmatory test of antimalarial activity in other tests. Rane and Rane (13) developed procedures with mosquitoes, *Aedes aegypti*, and sporozoite-induced infections of *Plasmodium gallinaceum* in chicks which allow successful testing of compounds for prophylactic activity. Rand and Rane stated that the 7-n-octadecylmercapto- and 7-n-tetradecylmercapto-6-hydroxy-5,8-quinolinequinone of Porter et al. (14) "demonstrated activity in the sporozoite screen," but gave no data.

Porter et al. (14) synthesized four new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones. Each of these analogs of coenzyme Q showed marked activity in vitro antimalarial activity without acute toxicity in the curative test against *P. berghei* in the mouse developed by Odsene et al. (15). Two of these compounds were curative, and one cured all mice in the treated group. These alkylmercapto-5,8-quinolinequinones, represented in the assay by three of the four, also showed marked inhibition of both mitochondrial CoQ-enzyme systems for NADH- and succinidase.

We have now synthesized, by improved conditions, seven additional and new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones and describe data on their antimalarial activities. In the sporozoite-induced assay developed by Rane and Rane (13), some of these antimitabolites of coenzyme Q showed effective prophylactic antimalarial activity in all of the chicks in the test group, and at a low dosage, and without toxicity as high as 30-fold dosage in comparison with the effective therapeutic dosage.

**MATERIALS AND METHODS**

All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra (Varian A-60) were taken on all new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones and were consistent with proposed structures. C, H, N analyses for each new quinone were within 0.04% of calculated values.

**General Procedure for 7-Alkylmercapto-6-hydroxy-5,8-quinolinequinones.** Seven new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones were synthesized by treating 6-hydroxy-5,8-quinolinequinone (16) in ethanol with the appropriate alkyl mercapta by a modification of the procedure described by Porter et al. (14). Generally, a solution of starting quinone was refluxed while the alkyl mercapta in hexane was added. In some reactions, a small amount of sulfuric acid was added. The resulting solutions were refluxed for 4–20 hr. and then cooled to room temperature. The solvents were removed by distillation in vacuo at room temperature. The resulting purple solid materials were redissolved in chloroform, and the solution was washed thoroughly with water. The chloroform was then removed, and the solid materials were washed with hexane. Finally, the products were recrystallized from chloroform-95% ethanol and/or 95% ethanol. When the desired product was noncrystalline, washing the crude product with both water and hexane became an essential step for the purification. The synthesis of crystalline 7-n-pentadecylmercapto-6-hydroxy-5,8-quinolinequinone, which is described in detail, is representative.

The synthesis of 7-phytylmercapto-6-hydroxy-5,8-quinolinequinone, which is described in detail, is representative of the noncrystalline quinone.

7-n-Pentadecylmercapto-6-hydroxy-5,8-quinolinequinone. 6-Hydroxy-5,8-quinolinequinone (5 g) was dissolved in 95% ethanol (300 ml). The solution was refluxed while n-pentadecyl mercapta (7 g) in hexane (20 ml) and 12 N sulfuric acid (3 ml) were added. The resulting solution was refluxed for 5 hr and then was cooled to room temperature. The solvents were removed at room temperature in vacuo, and chloroform was added. The chloroform solution was washed thoroughly with water and dried over anhydrous sodium sulfate. The chloroform was removed in vacuo, and the purple solid was washed with hexane to yield 3.3 g of product melting point, m.p. = 137–139.5°C. The analytical sample was prepared by recrystallization from chloroform-95% ethanol (1:9), m.p. = 138–139.5°C.

7-Phytalmercapto-6-hydroxy-5,8-quinolinequinone. 6-Hydroxy-5,8-quinolinequinone (4 g) was dissolved in 95% ethanol (300 ml). The solution was refluxed while phytal mercapta (3.8 g) in hexane (40 ml) was added. The resulting solution was refluxed for 20 hr. and then cooled to room temperature. The solvents were removed at room temperature, and chloroform was added. The chloroform solution was washed thoroughly with water and dried over anhydrous sodium sulfate. The chloroform was removed, and the purple waxy material was washed thoroughly with hexane to yield 0.5 g of product.

6-n-Dodecylmercapto-7-methoxy-5,8-quinolinequinone. 7-Hydroxy-5,8-quinolinequinone (4 g) (17) in MeOH was treated with a few milliliters of methanolic HCl. The reaction mixture was refluxed for several hours, reduced in volume, and then stirred overnight. After standing in the cold room, the reaction mixture was filtered to yield a solid material, which was placed in MeOH-EtO and treated with n-dodecyl mercapta (4 g). This reaction mixture was stirred 3 days at room temperature and then 3 days at 46°C. Solvent was removed in vacuo. EtO-HCl and Celite were added, and the heated mixture was filtered to yield a cherry-red filtrate. The filtrate was evaporated in vacuo to a syrop. Addition of hexane and cooling yielded a crude crystalline material. Repeated recrystallizations from hexane-EtO-MeOH yielded 400 mg of material, m.p. 61–64°C still contaminated with impurities. This material in EtO-hexane-acetone was placed on a silica gel column and eluted with EtO-hexane and then EtO-hexane-CHCl₃. Yield 44 mg; m.p. = 59–61°C.

7-n-Octadecylmercapto-6-acetoxy-5,8-quinolinequinone. A mixture of 7-n-octadecylmercapto-6-hydroxy-5,8-quinolinequinone (1.3 g), acetic anhydride (50 ml), and pyridine (10 drops) was allowed to stir overnight. The color of the solution turned from red to yellow, and a precipitate formed. The yellow precipitate was collected, and the filtrate was poured into about 100 ml of ice water. The resulting yellow precipitate was collected and combined with the product already collected. Recrystallization of the product from 95% ethanol gave 1.4 g of yellow crystals, m.p. = 91–92°C (96% yield).

**RESULTS**

**Organic Syntheses**

The synthesis of the new 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones was accomplished by treating 6-hydroxy-5,8-quinolinequinone in ethanol with the appropriate alkyl mercapta as depicted in Scheme II. n-Pentadecyl, n-heptadecyl, n-nonadecyl, and n-docosyl mercaptans were chosen, because they provided side chain lengths near the optimal length for maximal in vivo activity found in the series of 7-n-
alkyl-6-hydroxy-5,8-quinolinequinones by Porter et al. (18). \(\omega\)-Cyclohexylhexyl, oleyl, and phytanyl mercaptans provided branched, unsaturated, and isoprenoid side chains.

The yields of products were increased by raising the reaction temperature and by adding a small amount of sulfuric acid. However, for reactions involving unsaturated alkyl mercaptans, the addition of sulfuric acid was omitted. Each of the 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones, except 7-phytylmercapto-6-hydroxy-5,8-quinolinequinone, is a dark-purple crystalline compound with a relatively sharp melting point (Table 1).

Similarly, 6-n-dodecylmercapto-7-methoxy-5,8-quinolinequinone was prepared by treating 7-methoxy-5,8-quinolinequinone, 320, and 640 derivatives were obtained by treating the corresponding alcohols with phosphorus tribromide. The alkyl bromides were allowed to react with thiourea, and the thiouanium salts were hydrolyzed with sodium hydroxide to give the desired mercaptans.

**Procedures and data on curative and prophylactic antimalarial activities**

Representative compounds were tested for antimalarial activity against *Plasmodium berghei* (blood-induced) in mice (15) (Table 1), and for prophylactic activity against *P. gallinaceum* (sporozoite-induced) in chicks (13) (Table 2). Quinones 1, 2, 4, and 5 were tested against *P. berghei*. The data in Table 1 show that the heptadecylmercapto derivative (quinone 2) was active at 80 mg/kg, and the pentadecylmercapto derivative was active at 160 mg/kg and cured 2/5 mice at 640 mg/kg. For comparison, the previously published data of quinones 8, 9, 10, and 11 (14) are included. The dodecylmercapto and hexadecylmercapto derivatives were active at 320 mg/kg and 160 mg/kg, respectively. The octadecylmercapto derivative cured 5/5 mice at both 320 and 640 mg/kg. No deaths in mice were observed in the test of any of these seven quinones. It is particularly interesting that the octadecylmercapto derivative was active in 160 mg/kg and actually cured 3/5 mice, and that the docosylmercapto derivative differing by only four additional methylene groups was inactive at 160 mg/kg. This deceptively minor difference in

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>R = ((\text{CH}_2)_n\text{CH}_3)</th>
<th>m.p. °C</th>
<th>% Yield</th>
<th>(T - C^*) mg/kg</th>
<th>Cures at</th>
<th>Toxicity deaths at mg/kg</th>
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<tbody>
<tr>
<td>1</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>138-139.5</td>
<td>27.7</td>
<td>7.9 at 160</td>
<td>0/5 at 160</td>
<td>0/5 at 160</td>
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<td>2</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>137-138</td>
<td>35.9</td>
<td>12.9 at 640</td>
<td>2/5 at 640</td>
<td>0/5 at 640</td>
</tr>
<tr>
<td>3</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>136-137</td>
<td>21.1</td>
<td>1.3 at 10</td>
<td>0/5 at 10</td>
<td>0/5 at 10</td>
</tr>
<tr>
<td>4</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>134.5-136</td>
<td>32.9</td>
<td>2.5 at 20</td>
<td>0/5 at 20</td>
<td>0/5 at 20</td>
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<tr>
<td>5</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>147.5-148.5</td>
<td>42.4</td>
<td>4.1 at 40</td>
<td>0/5 at 40</td>
<td>0/5 at 40</td>
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<td>6</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>118-119</td>
<td>26</td>
<td>10.9 at 80'</td>
<td>0/5 at 80</td>
<td>0/5 at 80</td>
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<td>7</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>8.5</td>
<td></td>
<td>13.9 at 320</td>
<td>0/5 at 320</td>
<td>0/5 at 320</td>
</tr>
<tr>
<td>8</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>3.7 at 160</td>
<td>5.9 at 20</td>
<td>2/5 at 80</td>
<td>0/5 at 160</td>
<td>0/5 at 160</td>
</tr>
<tr>
<td>9</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>17.9 at 160</td>
<td>5.9 at 20</td>
<td>3/5 at 160</td>
<td>0/5 at 160</td>
<td>0/5 at 160</td>
</tr>
<tr>
<td>10</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>9.5 at 160</td>
<td>5/5 at 640</td>
<td>3/5 at 320</td>
<td>0/5 at 320</td>
<td>0/5 at 320</td>
</tr>
<tr>
<td>11</td>
<td>((\text{CH}_2)_n\text{CH}_3)</td>
<td>5/5 at 640</td>
<td></td>
<td>5/5 at 640</td>
<td>0/5 at 640</td>
<td>0/5 at 640</td>
</tr>
</tbody>
</table>

*a* Nuclear magnetic resonance spectra were obtained in DCCl₃ with a Varian Associates A-60 spectrometer and were consistent with proposed structures.

*b* C, H, N analyses for each new quinone were within 0.40% of calculated values.

All compounds were administered subcutaneously, in graded doses, to groups of five mice.

Yields are based on starting alkyl mercuran.

\(T - C\) = Change in survival time, in days, of treated and nontreated (control) mice. Control mice survived an average of 6.2 days.

The compound was declared "active" at this dose level by the standard of 100% increase in survival time.

"Active" compounds were those that showed a significant difference in mortality compared to the control group.

The synthesis and assay data for this compound have been published previously (14).

Mass spectrum of this compound indicated trace impurities.
structure between the octadecylmercapto and docosylmercapto derivatives is biologically profound and presumably correlates with precise requirements of the enzyme sites for the functionality and/or biosynthesis of coenzyme QA in the parasite.

For the sporozoite-induced and prophylactic chick tests, the data are in Table 2. For comparison, the previously synthesized tetradecylmercapto, hexadecylmercapto, and octadecylmercapto derivatives are included in Table 2, because assay data are now available. The prophylactic antimalarial activity in this assay is not only effective but prominent in this group of 7-alkylmercapto-6-hydroxy-5,8-quinolines. The heptadecylmercapto derivative (quinone 2) cured 5/5 mice at only 10 mg/kg and is presumably active at even lower dose levels. The absence of toxicity at 320 mg/kg demonstrates an exceptional margin of safety. The octadecylmercapto derivative showed only marginal activity at 100 mg/kg, and the nonadecylmercapto derivative was active but less active than the heptadecylmercapto derivative. Consequently, increasing the chain length beyond seventeen carbon atoms reduced activity. Shortening the chain length to the hexadecylmercapto derivative resulted in 5/5 cures at 120 mg/kg, which was the lowest dosage tested. The tetradecylmercapto derivative showed diminished activity in comparison with the heptadecylmercapto derivative. It appears that maximal activity of these 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones resides in those compounds having 15, 16, and 17 carbon atoms in their saturated straight alkyl groups. Quinone 6, possessing one double bond in the 7-alkylmercapto group, exemplifies the unexplored potentiality of even better activity, since unsaturation is natural in the isoprenoid side chain of coenzyme QA. The octadecylmercapto analog showed 1/5 cures at 40 mg/kg in comparison with the oleylmercapto analog with 1 unit of unsaturation, which showed 4/5 cures at 15 mg/kg, apparently, unsaturation in this alkyl side chain enhances prophylactic activity.

7-n-Octadecylmercapto - 6-acetoxy - 5,8-quinolinequinone (quinone 12) cured 2/5 mice at 80 mg/kg, 4/5 mice at 160 mg/kg, and 5/5 mice at 320 mg/kg in the prophylactic chick assay. No toxicity was evident at 320 mg/kg for the 6-acetoxy

### Table 2. Antimalarial activity of certain alkylmercaptoquinones in the sporozoite-induced chick test (13)

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Structure</th>
<th>In vivo antimalarial activity, chick test* (P. gallinaceum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T - C, mg/kg</td>
</tr>
<tr>
<td>9*</td>
<td>R = -(CH2)13CH3</td>
<td>7.6 at 20</td>
</tr>
<tr>
<td>10*</td>
<td>R = -(CH2)15CH3</td>
<td>3.5 at 40</td>
</tr>
<tr>
<td>2</td>
<td>R = -(CH2)16CH3</td>
<td>0.3 at 15</td>
</tr>
<tr>
<td>11*</td>
<td>R = -(CH2)17CH3</td>
<td>1.7 at 40</td>
</tr>
<tr>
<td>3</td>
<td>R = -(CH2)19CH3</td>
<td>0.9 at 15</td>
</tr>
<tr>
<td>6</td>
<td>R = -(CH2)CH==CH(CH2)15CH3</td>
<td>1.7 at 80</td>
</tr>
<tr>
<td>12*</td>
<td>OAc</td>
<td>3.0 at 320</td>
</tr>
</tbody>
</table>

* All compounds were administered subcutaneously to groups of five chicks.
* T - C = Change in survival time, in days, of treated and nontreated (control) chicks.
* This compound has been previously synthesized (14).
* The compound was declared “active” by the standard of 100% increase in survival time.
* C, H, N analyses for compound 12 were within 0.40% of calculated values.
quinoine, but the corresponding 6-hydroxy quinone showed 2/5 toxic deaths at 320 mg/kg.

3 - n - Dodecylmercapto - 2 - hydroxy - 1,4 - naphthoquinone (quinone 13) was inactive in the chick assay. The pyridine moiety of the quinolinequinone system is apparently important for the inhibitory role in these quinones, possibly because of its influence on the oxidation-reduction potential and/or binding at receptor sites.

Initial tests in two rhesus monkeys showed suppression of P. cynomolgi when the 7-octadecylmercapto derivative was intravenously administered at a dosage of 3.16 mg/kg per day for 7 consecutive days. When this compound was given intraperitoneally at the same dose level (3.16 mg/kg per day), one of two monkeys died of drug toxicity; the monkey that survived the drug toxicity showed no beneficial effects of the compound given. Intravenous administration at 10 mg/kg per day produced toxic deaths in two of three monkeys; intraperitoneal administration at 10 mg/kg per day produced peritonitis and/or drug toxicity so severe that 4/4 monkeys died by day 15. This compound was completely ineffective by the oral route of administration at 31.6 mg/kg and 10 mg/kg. The compound was given at each dosage level to two monkeys for 7 consecutive days. No toxicity was seen.

**DISCUSSION**

The structural comparison (Scheme III) of the heptadecylmercapto derivative, 2, and coenzyme Q₈ shows the potentially competitive relationship for inhibition of functionality in the electron transfer mechanism of the parasite. The comparison of the heptadecylmercapto derivative with the three precursors 14, 15, and 16, which are quinones in the projected biosynthesis (19) of coenzyme Q₈ by the parasite, shows the potentiality of additional sites of inhibition for antimalarial activity.

Although antimetabolic activity or inhibition of coenzyme Q enzymes has been demonstrated (14) for these particular quinones, the biochemical basis of the antimalarial activity, in vivo, can be the resultant of single or multiple inhibition(s) of the functionality and/or the biosynthesis of coenzyme Q₈ as well as other metabolic reactions.

The assays of these quinones against P. berghei in mice and P. gallinaceum in chicks is only a screening system, albeit a very efficient one. As such, the test compounds are administered only once. Consequently, the effectiveness of the heptadecylmercapto derivative in the prophylactic chick test is indeed impressive. It may be assumed on the basis of conventional chemotherapy that multiple dosage, in vivo, could yield data even more impressive than that presently available on the single-dosage schedule.

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