

## In Vitro Increase in Chloroquine Accumulation Induced by Dihydroethano- and Ethenoanthracene Derivatives in *Plasmodium falciparum*-Parasitized Erythrocytes

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**The effects of a series of dihydroethano- and ethenoanthracene derivatives on chloroquine (CQ) accumulation in CQ-susceptible strain 3D7 and CQ-resistant clone W2 were assessed. The levels of CQ accumulation increased little or none in CQ-susceptible strain 3D7 and generally increased markedly in CQ-resistant strain W2. At 10  $\mu$ M, 28 compounds yielded cellular accumulation ratios (CARs) greater than that observed with CQ alone in W2. At 10  $\mu$ M, in strain W2, 21 of 31 compounds had CQ CARs two or more times higher than that of CQ alone, 15 of 31 compounds had CQ CARs three or more times higher than that of CQ alone, 13 of 31 compounds had CQ CARs four or more times higher than that of CQ alone, and 9 of 31 compounds had CQ CARs five or more times higher than that of CQ alone. At 1  $\mu$ M, 17 of 31 compounds had CQ CARs two or more times higher than that of CQ alone, 12 of 31 compounds had CQ CARs three or more times higher than that of CQ alone, 6 of 31 compounds had CQ CARs four or more times higher than that of CQ alone, and 3 of 31 compounds had CQ CARs five or more times higher than that of CQ alone. At 1  $\mu$ M, 17 of 31 compounds were more potent inducers of CQ accumulation than verapamil and 12 of 31 compounds were more potent inducers of CQ accumulation than promethazine. The nature of the basic group seems to be associated with increases in the levels of CQ accumulation. At 1 and 10  $\mu$ M, 10 of 14 and 13 of 14 compounds with amino group (amines and diamines), respectively, had CARs  $\geq 3$ , while at 1 and 10  $\mu$ M, only 1 of the 13 derivatives with amido groups had CARs  $\geq 3$ . Among 12 of the 31 compounds which were more active inducers of CQ accumulation than promethazine at 1  $\mu$ M, 10 had amino groups and 1 had an amido group.**

Chloroquine (CQ) has been one of the most successful antimalarial drugs ever developed. During the past 20 years, strains of *Plasmodium falciparum* have become resistant to CQ and other antimalarial drugs (49, 57). The universal success of CQ before the development of resistance has led to investigations of the mode of action of CQ and its mechanisms of resistance. CQ reaches the parasite food vacuole, where it accumulates due to the local acid pH and the weak base properties of the drug (59). The activity of CQ depends on a high level of accumulation of the drug within the parasite, and drug resistance is indicated by reduced levels of drug accumulation.

Some interesting observations suggest that the mechanism of malarial CQ resistance may be similar to the mechanism of mammalian multidrug resistance (MDR). In cancer cell lines, the resistance-reversing actions of chemosensitizing drugs have been fairly well characterized. MDR is defined as the MDR conferred on cell lines by increased levels of expression of the human MDR-1 protein (P-glycoprotein) or closely related animal equivalents (41). Resistant parasites actively expel CQ (35), probably by means of a transporter encoded by an MDR gene (19). Two genes, *pfmdr1* and *pfmdr2*, that are homologues of the mammalian MDR genes were cloned from *P. falciparum*

(27, 60). Pgh-1, the product of *pfmdr1*, was found to be localized in the food vacuole membrane (19), suggesting that it could be involved in drug transport across this membrane (33). It was initially suggested that Pgh-1 pumps CQ out of the food vacuole and is expressed in CQ-resistant parasites (35). Nevertheless, the level of resistance does not correlate with the level of Pgh-1 expression (58). In addition, selection of CQ resistance is associated with a decrease in the number of copies of *pfmdr1* (4). Another possibility could be that Pgh-1 pumps CQ into the food vacuole. This notion would agree with the idea that drug resistance may be due to mutations in *pfmdr1*. It is incompatible with the demonstration that the gene for the CQ resistance phenotype does not segregate with *pfmdr1* in a genetic cross of CQ-susceptible and CQ-resistant strains (56). The presence of a mutation in the tyrosine-86 allele was considered to be associated with CQ resistance (31). However, epidemiological studies conducted with a larger number of samples to provide a meaningful analysis found no correlation between a mutation in the tyrosine-86 allele and CQ resistance (7). Several studies failed to show any association between a mutation in Pgh-1 and CQ resistance (12). Although Pgh-1 may act as a drug pump, it does not seem to be involved in resistance to CQ. Pgh-1 might act as a chloride channel or as a modulator of such a channel (52). As a chloride channel, Pgh-1 may be constitutively expressed in the vacuolar membrane to allow the maximal conversion of the proton motive force of the vacuolar H<sup>+</sup> pump to achieve an acid pH. The pH

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of the food vacuole affects the level of drug accumulation by proton trapping, as well as hemoglobin digestion and the crystallization of ferriprotoporphyrin into hemozoin, both of which are pH-dependent activities (45). A lower pH would lead to enhanced CQ accumulation and drug susceptibility, and drug resistance could therefore result from mutations in Pgh-1.

It has recently been shown that CQ resistance in a *P. falciparum* cross maps to a 36-kb segment of chromosome 7 (46). This segment accommodates *cg2*, a gene that encodes a unique protein which has been detected in the parasite cytosol, the parasitophorous space, and the food vacuole in association with hemozoin. This Cg2 molecule could therefore be implicated in CQ transport and in the inhibition of ferriprotoporphyrin IX polymerization. Polymorphisms in *cg2* were highly associated with CQ resistance (8), but allelic modification experiments have ruled out a role for this gene in CQ resistance (25).

Recently, *pfct*, a gene with 13 exons, was identified near *cg2* on chromosome 7 (26). This transmembrane protein localizes to the parasite digestive vacuole, the site of CQ action, where increased acidification of the compartment is associated with a point mutation in *pfct* (26). One mutation at position 76 was present in all resistant isolates and was absent from all susceptible isolates. *pfct* genotypes are strongly linked with in vitro and in vivo CQ resistance (3, 9, 18, 20, 22). The mutation in the threonine-76 allele of the protein encoded by *pfct* can be used as a marker in surveillance for CQ-resistant falciparum malaria. Mutations in *Pfct* appear to be associated with changes in vacuolar pH (26). Models of CQ resistance that incorporate the effects of mutations in *pfct* in different ways can be envisaged: the decrease in the vacuolar pH associated with mutations in *pfct* reduces the drug-heme interaction responsible for toxicity, and drug flux across the membrane of the digestive vacuole is directly altered by a structural change in the *Pfct* molecule itself or by an effect of *Pfct* on the functions of other molecules involved in the physiology of the digestive vacuole. Changes in pH are reported to have a strong effect on the conversion of soluble heme to insoluble aggregates (21, 23). The midpoint pH of this conversion is close to the vacuolar pH values of CQ-resistant parasites (50). The formation of insoluble heme is much more efficient at the more acid vacuolar pH values of CQ-resistant parasites. Acidification of the digestive vacuole would leave significantly less free heme available for the formation of toxic complexes with CQ.

The reversal of CQ resistance by compounds with little intrinsic antimalarial activities may become a chemotherapeutic alternative (38). Several compounds like verapamil (1, 35, 39, 43), desipramine (5, 6, 13, 17), and antihistaminic drugs (10, 40, 42) have been demonstrated in the past decade to have promising capabilities to reverse the CQ resistance in parasite isolates in vitro and in animal models (36, 40, 47). The reversal of CQ resistance by verapamil was proposed to occur by modulating the activity of the parasitic  $\text{Na}^+\text{-H}^+$  exchanger via the calcium- and calmodulin-dependent pathway (44). Nevertheless, Bray et al. (16) provided definitive evidence that CQ uptake is determined by the binding of CQ to ferriprotoporphyrin IX. Recently, it has been shown that the digestive vacuole of a CQ-resistant strain is more acidic than the digestive vacuoles of CQ-susceptible parasites (23). Verapamil normalizes the vacuolar pH of the CQ-resistant parasites to a value

near that measured for CQ-susceptible parasites (by increasing the pH) and has no effect on CQ-susceptible parasites (51). In fact, it seems that acidification of the digestive vacuole contributes to drug resistance via the effects that the pH has on the solubility of unpolymerized heme found in the vacuole (50). Reversers of CQ resistance increase the pH of acid vesicles where ferriprotoporphyrin IX is generated, and this increased pH therefore increases the affinity of CQ-ferriprotoporphyrin IX binding.

We previously synthesized and evaluated three dihydroethano- and ethenoanthracene compounds which showed synergistic activities when they were used in combination with CQ against CQ-resistant parasites (2). The aim of this study was to assess the capacities of these 3 compounds and 28 other analogue derivatives to increase the level of accumulation of CQ in CQ-resistant *P. falciparum* parasites.

## MATERIALS AND METHODS

**Strains of *P. falciparum*.** CQ-resistant clone W2 (from Indochina) and CQ-susceptible strain 3D7 (from Africa) were maintained in culture. When required for the drug assays, the cultures were synchronized by sorbitol lysis (37).

**Drugs.** The synthesis of some of the dihydroanthracene derivatives used in this study was described previously (2, 28, 34).

Verapamil and promethazine were obtained from Sigma Chemical (St. Louis, Mo.). The drugs used for the reversal of resistance (referred to as reversal drugs; 1 and 10  $\mu\text{M}$ ) were prepared in RPMI 1640 medium (Life Technologies, Paisley, United Kingdom) buffered with 25 mM HEPES and 25 mM  $\text{NaHCO}_3$  containing 3 nM labeled CQ. Silicon oil 550 was purchased from Dow Corning (BDH Laboratory Supplies, Poole, United Kingdom). Radiolabeled CQ diphosphate ( $^3\text{H}$ CQ; 26 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Little Chalfont, United Kingdom).

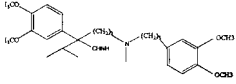
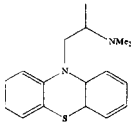
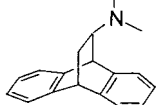
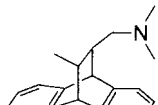
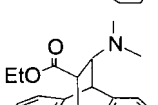
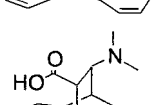
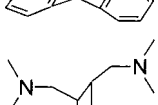
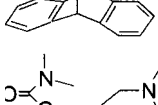
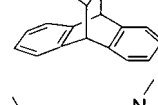
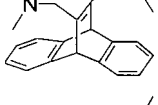
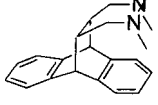
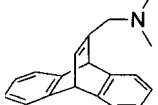
The lipophilicities of the drugs tested were expressed by calculation of the log *P* value (determined with Pallas software [version 2.0; CompuDrug Chemistry Ltd.]).

**Measurement of  $^3\text{H}$ CQ uptake by parasitized erythrocytes.** The level of accumulation of  $^3\text{H}$ CQ was measured essentially by the protocol of Bray et al. (16). Infected erythrocytes with a level of parasitemia of 2% and a hematocrit of 2% were suspended in RPMI 1640 medium buffered with 25 mM HEPES and 25 mM  $\text{NaHCO}_3$ . Eppendorf microcentrifuge tubes were loaded with 400  $\mu\text{l}$  of silicon oil 550, 1 ml of reaction buffer containing  $^3\text{H}$ CQ and reversal drugs on top of the oil, and then 25  $\mu\text{l}$  of an appropriately concentrated cell suspension with a hematocrit of 75%. The cell suspension was mixed with the reaction buffer, and the mixture was incubated for 1 h at 37°C in an atmosphere of 10%  $\text{O}_2$ -6%  $\text{CO}_2$ -84%  $\text{N}_2$  with 95% relative humidity. After 1 min of centrifugation at 13,000  $\times$  g, 100  $\mu\text{l}$  of the buffer was processed for scintillation counting. The infected erythrocyte pellets were washed with distilled water, lysed with quaternary hydroxide-glacial acetic acid-hydrogen peroxide (5:5:2), and left in an oven at 50°C for 2 h. They were then processed for scintillation counting. The level of CQ accumulation is expressed as the cellular accumulation ratio (CAR), which is the ratio of the amount of radiolabeled CQ in parasites (amount of  $^3\text{H}$ CQ in parasitized erythrocytes - amount of  $^3\text{H}$ CQ in uninfected erythrocytes) to the amount of  $^3\text{H}$ CQ in a similar volume of buffer after incubation (14). The same procedure was applied to uninfected erythrocytes from the same source as the erythrocytes used in the *P. falciparum* culture.

## RESULTS

The series of dihydroanthracene derivatives had intrinsic antimalarial activities in vitro at concentrations that ranged from 2 to >500  $\mu\text{M}$ . The CARs of CQ are summarized in Table 1. The levels of CQ accumulation increased little or none in CQ-susceptible strain 3D7 and generally increased markedly in CQ-resistant strain W2. At 10  $\mu\text{M}$ , 28 compounds yielded CARs greater than those observed with CQ alone in W2. For statistical significance, it was considered that only compounds with CARs two or more times higher than that of

TABLE 1. In vitro activities of a series of dihydroethano- and ethaneanthracene derivatives against the CQ-resistant *P. falciparum* strain W2 and their effects on CQ CARs for CQ-susceptible and -resistant parasites

Structure <sup>a</sup>	Compound	Log <i>P</i>	IC <sub>50</sub> (μM) for W2 <sup>b</sup>	CQ CAR <sup>c</sup>			
				Clone 3D7		Clone W2	
				1 μM	10 μM	1 μM	10 μM
	CQ alone	4.7	0.9	209		17	
	Verapamil	3.8	13.2	247 (1.2)	203 (1.0)	16 (1)	97 (6)
	Promethazine	3.9	18.5	177 (0.8)	164 (0.8)	37 (2)	42 (2.5)
	BG916	3.7	11.9	237 (1.0)	329 (1.6)	49 (3)	216 (13)
	BG917	4.2	21.1	320 (1.5)	296 (1.4)	46 (3)	62 (4)
	BG918	3.6	29.9	279 (1.3)	216 (1.0)	62 (4)	63 (4)
	BG919	2.7	>500	213 (1.0)	195 (0.9)	18 (1)	16 (1)
	BG920	3.4	4.3	284 (1.4)	275 (1.3)	112 (7)	298 (18)
	BG921	3.0	21.2	337 (1.6)	312 (1.5)	50 (3)	66 (4)
	BG930	3.3	2.5	223 (1.1)	247 (1.2)	61 (4)	242 (14)
	BG932	3.4	2.1	200 (1.0)	270 (1.3)	76 (5)	217 (13)
	BG954	3.7	4.0	250 (1.2)	272 (1.3)	31 (2)	53 (3)
	BG955	2.2	35.9	235 (1.1)	244 (1.2)	30 (2)	23 (1)

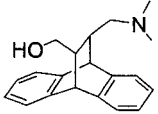
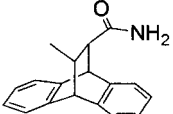
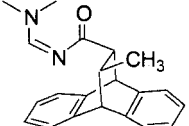
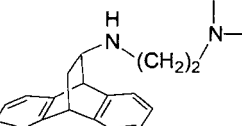
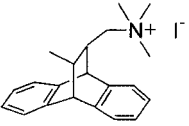
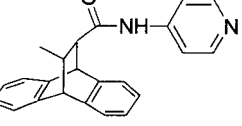
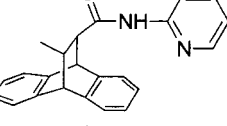
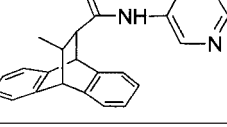
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TABLE 1—Continued

Structure <sup>a</sup>	Compound	Log P	IC <sub>50</sub> (μM) for W2 <sup>b</sup>	CQ CAR <sup>c</sup>			
				Clone 3D7		Clone W2	
				1 μM	10 μM	1 μM	10 μM
	BG956	1.7	37.2	258 (1.2)	235 (1.1)	28 (2)	31 (2)
	BG957	1.7	12.3	275 (1.3)	248 (1.2)	30 (2)	32 (2)
	BG958	3.0	11.7	294 (1.4)	203 (1.0)	89 (5)	322 (19)
	BG973	4.4	52.4	230 (1.1)	193 (0.9)	46 (3)	34 (2)
	BG991	9.2	8.2	231 (1.1)	224 (1.1)	27 (2)	34 (2)
	BG992	0.9	41.6	207 (1.0)	210 (1.0)	22 (1)	29 (2)
	BG993	1.9	7.8	240 (1.1)	198 (0.9)	50 (3)	159 (9)
	BG995	3.8	40.0	229 (1.1)	216 (1.0)	19 (1)	22 (1)
	BG996	3.7	8.0	242 (1.2)	228 (1.1)	68 (4)	210 (12)
	BG997	1.6	33.7	239 (1.1)	234 (1.1)	16 (1)	25 (1)
	BG998	2.6	34.9	207 (1.0)	198 (0.9)	17 (1)	22 (1)
	BG999	3.0	32.1	198 (0.9)	205 (1.0)	20 (1)	19 (1)
	BG1000	2.5	33.3	244 (1.2)	235 (1.1)	23 (1)	30 (2)

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TABLE 1—Continued

Structure <sup>a</sup>	Compound	Log P	IC <sub>50</sub> (μM) for W2 <sup>b</sup>	CQ CAR <sup>c</sup>			
				Clone 3D7		Clone W2	
				1 μM	10 μM	1 μM	10 μM
	BG1001	2.8	35.2	267 (1.3)	279 (1.3)	14 (1)	56 (3)
	BG1002	2.9	31.6	185 (0.9)	200 (1.0)	17 (1)	22 (1)
	BG1003	3.3	49.0	255 (1.2)	244 (1.2)	23 (1)	19 (1)
	BG1004	3.2	8.2	300 (1.4)	243 (1.2)	16 (1)	71 (4)
	BG1014	1.3	16.6	341 (1.6)	300 (1.4)	53 (3)	192 (11)
	BG1049	3.6	>500	171 (0.8)	305 (1.5)	15 (1)	101 (6)
	BG1050	3.8	214.3	159 (0.8)	160 (0.8)	14 (1)	15 (1)
	BG1051	3.6	78.8	186 (0.9)	237 (1.1)	10 (1)	10 (1)

<sup>a</sup> Me, methyl; Et, ethyl; Ph, phenyl.

<sup>b</sup> IC<sub>50</sub>s for W2 are means of 4 to 12 independent experiments.

<sup>c</sup> The values in parentheses are CQ CARs for CQ and the compound/CQ CAR of CQ alone.

CQ increased the level of accumulation of CQ. At 10 μM, in W2, 21 of 31 compounds had CQ CARs two or more times higher than that of CQ alone, 15 of 31 compounds had CQ CARs three or more times higher than that of CQ alone, 13 of 31 compounds had CQ CARs four or more times higher than that of CQ alone, and 9 of 31 compounds had CQ CARs five or more times higher than that of CQ alone (Table 1). At 1 μM, 17 of 31 compounds had CQ CARs two or more times higher than that of CQ alone, 12 of 31 compounds had CQ CARs three or more times higher than that of CQ alone, 6 of 31 compounds had CQ CARs four or more times higher than

that of CQ alone, and 3 of 31 compounds had CQ CARs five or more times higher than that of CQ alone. At 1 μM, 17 of 31 compounds were more potent inducers of CQ accumulation than verapamil and 12 of 31 compounds were more potent inducers of CQ accumulation than promethazine. The nature of the basic group seems to be associated with increases in the levels of CQ accumulation. At 1 and 10 μM, 10 of 14 and 13 of 14 compounds with amino group (amines and diamines), respectively, had CARs ≥3 (Table 2). At 1 and 10 μM, only 1 of 13 derivatives with amido groups had CARs ≥3. Among 17 of the 31 compounds which were more potent inducers of CQ

TABLE 2. Effects at 1 and 10  $\mu\text{M}$  of dihydroethano- and ethaneanthracene derivatives with substituted groups on CARs of CQ

Substituted group	Drug	No. of compounds with CARs $\geq 3$ /total no.	
		1 $\mu\text{M}$	10 $\mu\text{M}$
Secondary amine	BG958, BG996	2/2	2/2
Tertiary amine	BG916, BG917, BG954	2/3	3/3
Tertiary amine and alcohol	BG1001	0/1	1/1
Tertiary amine and carboxylic acid	BG919	0/1	0/1
Tertiary amine and ester	BG918	1/1	1/1
Tertiary amine and carbamate	BG921	1/1	1/1
Diamine	BG1004	0/1	1/1
Primary diamine	BG993	1/1	1/1
Tertiary diamine	BG920, BG930, BG932	3/3	3/3
Primary amide	BG998, BG1002	0/2	0/2
Secondary amide	BG973, BG1049, BG1050, BG1051	1/4	1/4
Tertiary amide	BG999	0/1	0/1
Tertiary amide and ester	BG1000	0/1	0/1
Primary diamide	BG992	0/1	0/1
Secondary diamine	BG991	0/1	0/1
Tertiary diamide	BG955, BG956, BG957	0/3	0/3
Quaternary ammonium	BG1014	1/1	1/1
Carbamidine	BG1003	0/1	0/1
Dicarbamidine	BG997	0/1	0/1
Bromide and carboxylic acid	BG995	0/1	0/1

accumulation than verapamil at 1  $\mu\text{M}$ , 11 had amino groups and 5 had amido groups. Among 12 of 31 compounds which were more active inducers of CQ accumulation than promethazine at 1  $\mu\text{M}$ , 10 had amino groups and 1 had an amido group. The CARs obtained at concentrations of 1 and 10  $\mu\text{M}$  are not correlated with lipophilicity ( $r = 0.093$  and  $0.011$ , respectively).

## DISCUSSION

The activity of CQ depends on a high level of accumulation of the drug within the parasite, and drug resistance stems from reduced levels of drug accumulation. Differentials in uptake versus efflux of CQ by CQ-susceptible and CQ-resistant parasites have both been proposed as explanations for the lower levels of accumulation of CQ by CQ-resistant parasites, but the debate has not yet been settled (11, 15, 30, 54). Some investigators have concluded that drug resistance can be accounted for by a decrease in the level of CQ uptake by CQ-resistant parasites (15, 24, 54), while others believe that CQ-resistant parasites appear to counter the drug by expelling it rapidly via a pump-mediated mechanism (35).

We detected a 12-fold difference in the level of CQ accumulation between CQ-susceptible strain 3D7 and CQ-resistant W2 strain, which is consistent with previous findings (16, 39, 48). As expected, with verapamil and promethazine, no significant alteration in the levels of CQ uptake was observed in CQ-susceptible strain 3D7 (16, 39, 53, 55). The level of CQ accumulation increased little in strain 3D7 in assays with the 31 compounds, as expected. Our results are consistent with those of other reports demonstrating increased levels of CQ accumulation in the presence of verapamil and a single low extracellular concentration of CQ. Verapamil at 10  $\mu\text{M}$  is able to increase the level of CQ uptake sixfold over that for the parasite control for CQ-resistant parasites, while at concentrations ranging from 2 to 10  $\mu\text{M}$ , it increases the level of CQ accumulation two- to fivefold, as determined in previous works (14,

16, 32, 39, 55). Large increases in the levels of CQ accumulation in CQ-resistant parasites were detected for 15 compounds tested at 10  $\mu\text{M}$  and 12 compounds tested at 1  $\mu\text{M}$ , with CARs being  $\geq 3$ , indicating that these compounds are more potent inducers of CQ accumulation than verapamil and promethazine. Lipophilicity does not seem to be important for the increase in the level of CQ accumulation in parasites. While most chemosensitizers bear little structural similarity to one another, several essential components are believed to play a role in the reversal of resistance. These include the presence of benzene groups, a secondary or tertiary nitrogen, and a cationic charge (29, 48). Most of our compounds have these characteristics. However, the presence of a secondary or a tertiary nitrogen is not enough to increase the level of CQ accumulation. The nature of the basic group seems to be associated with increases in the levels of CQ accumulation. At 1 and 10  $\mu\text{M}$ , 10 of 14 and 13 of 14 compounds with amino group (amines and diamines), respectively, induced increases in the levels of CQ accumulation (CARs,  $\geq 3$ ), while only 1 of 13 of the derivatives with an amido group gave the same result. Among the 12 of 31 compounds which were more active inducers of CQ accumulation than promethazine at 1  $\mu\text{M}$ , 10 had amino groups and 1 had an amido group. The presence of a secondary or a tertiary nitrogen is required to increase the level of CQ accumulation in CQ-resistant parasites, and the presence of one or two amino groups is especially required.

Some of these compounds, such as BG917, BG954, BG958 or BG920, and BG996, have previously been shown to have synergistic effects in combination with CQ (2; B. Pradines et al., submitted for publication). BG958, BG920, and BG996 were the most potent inducers of increases in CQ activity against CQ-resistant parasites. These three amino derivatives had CARs  $\geq 4$  when they were tested at 1  $\mu\text{M}$  and CARs  $\geq 12$  when they were tested at 10  $\mu\text{M}$ . BG917, whose ability to potentiate CQ activity is better than that of verapamil, had CARs between 3 and 4 when it was tested at 1 and 10  $\mu\text{M}$ .

BG954, which is a less potent inducer of CQ activity than verapamil, had CARs  $\leq 3$  when it was tested at 1 and 10  $\mu\text{M}$ . The time has come to systematically relate all of the CQ CARs to the pharmacodynamic interaction between CQ and the compounds tested and to assess whether there is a correlation between the synergistic effects of the compounds and CQ on the activity of CQ and their capacities to increase the level of CQ accumulation.

These compounds, which possess a protonatable nitrogen at physiological pH, could act by ionic interactions with three potential targets: Pgh-1, Cg2, and Pfert. Our compounds with the ability to reverse CQ resistance are structural analogues of CQ. Our first hypothesis was to suppose that these derivatives might enhance the level of CQ accumulation and improve the ability of CQ to access ferriprotoporphyrin IX by binding in a competitive way to a CQ transmembrane transporter such as Pgh-1 or Pfert. This increased level of accumulation of CQ could be the result of the higher affinities of the dihydroethano- and ethenoanthracene compounds for the export transporter. The reversal mechanism would be assumed to result from competition between our derivatives and CQ for efflux translocation sites, thus causing an increase in the steady-state level of accumulation of CQ and hence a return to susceptibility. However, our data do not directly support the conclusion that dihydroethano- and ethenoanthracene compounds interact with proteins such as Pgh-1 or Pfert, and it is not yet known if our compounds directly compete for a CQ-binding site on drug transporters involved in *P. falciparum* resistance.

Assessment of the effects of the compounds tested on the level of CQ accumulation allowed the rapid screening of compounds which could show synergistic effects in combination with CQ. Some of these dihydroethano- and ethenoanthracene derivatives hold much promise as effective antimalarial agents for use in combination with CQ. It is therefore important to identify the target of these compounds and to assess their in vitro activities in combination with CQ, their in vitro toxicities, and their efficacies in animal models.

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