Antimalarial Dual Drugs Based on Potent Inhibitors of Glutathione Reductase from *Plasmodium falciparum*

Wolfgang Friebolin,[§] Beate Jannack,[§] Nicole Wenzel,[§] Julien Furrer,[†] Thomas Oeser,[†] Cecilia P. Sanchez,[#] Michael Lanzer,[#] Vanessa Yardley,[¶] Katja Becker,[⊥] and Elisabeth Davioud-Charvet*[,§,‡]

Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 504, 69120 Heidelberg, Germany, Organisch-Chemisches Institut der Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany, Hygiene Institut, Abt. Parasitologie, Im Neuenheimer Feld 324, 69120 Heidelberg, Department of Infections and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K., Interdisciplinary Research Center, Justus-Liebig-University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, and Centre National de la Recherche Scientifique, 3 Rue Michel-Ange, 75794 Paris Cedex 16, France

Received July 30, 2007

Plasmodium parasites are exposed to higher fluxes of reactive oxygen species and need high activities of intracellular antioxidant systems providing a steady glutathione flux. As a future generation of dual drugs, 18 naphthoquinones and phenols (or their reduced forms) containing three different linkers between the 4-aminoquinoline core and the redox active component were synthesized. Their antimalarial effects have been characterized in parasite assays using chloroquine-sensitive and -resistant strains of *Plasmodium*, alone or in drug combination, and in the *Plasmodium berghei* rodent model. In particular, two tertiary amides **34** and **36** showed potent antimalarial activity in the low nanomolar range against CQ-resistant parasites. The ability to compete both for (Fe^{III})protoporphyrin and for chloroquine transporter was determined. The data are consistent with the presence of a carrier for uptake of the short chloroquine analogue **2** but not for the potent antimalarial amide **34**, suggesting a mode of action distinct from chloroquine mechanism.

Introduction

During the past decades chloroquine (CQ[a]) was one of the most successful antimalarial drugs since its introduction 60 years ago. This success was based on both its high antimalarial effectiveness and its cheapness and safety. It was shown that the antimalarial 4-aminoquinolines, like CQ, accumulate in the acidic food vacuole of the parasites and inhibit heme biomineralization.[1–6] Consequently toxic heme builds up in the vacuole, subsequently killing the parasite. However, the use of CQ as a standard therapeutic drug became more and more restricted in the world because of the dissemination of resistant malaria strains of which *Plasmodium falciparum* is the most deadly species. CQ resistance in *Plasmodium falciparum* is associated with mutations in the digestive vacuole transmembrane protein PfCRT[7–11] and with elevations of intracellular glutathione concentrations.[12] *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) belongs to the drug/metabolite superfamily[13,14] and is thought to act as a channel[15–17] or a carrier of chloroquine.[7,9,17–22]

Evidence from structure–activity relationships in the 4-aminoquinoline series has demonstrated that CQ resistance can be overcome by subtly altering the length[23–26] and the basic nature of the CQ side chain.[27] The level of accumulation depends upon the pK_a of the quinoline side chain nitrogen.[28,29] However, CQ analogues with a shortened *N*-alkyl side chain, although very active against sensitive as well as resistant malarial strains in vitro, were less active in vivo tests because of rapid N-dealkylation in the parasite/cell.[26] Modifications of the substituents on the aromatic nucleus,[24,25,30,31] e.g., exchange of the 7-chloro atom, or the deaza-bioisosteres of CQ[32,33] have only little influence toward resistant strains or are inactive. In addition, introduction of structural diversity in the side chain of 4-aminoquinolines was recently shown to enhance activity against drug-resistant *P. falciparum*[34,35] or to affect the partition coefficient leading to distinct localization of the 4-aminoquinoline in the parasites.

Plasmodium parasites are exposed to higher fluxes of reactive oxygen species and need high activities of intracellular antioxidant systems. They do not develop in glucose-6-phosphate dehydrogenase deficient red blood cells or in erythrocytes depleted in glutathione reductase activity.[36,37] High flux of reactive oxygen species results both from the host immune response to infection and from hemoglobin digestion. In sensitive parasites, CQ prevents heme detoxification, resulting in free heme accumulation and consequently in parasite death and erythrocyte lysis. Free heme in concert with oxygen species is thought to catalyze oxidation reactions and protein damage. The small amount of free heme (Fe^{3+}) released from the food vacuole in the cytosol is rapidly reduced into heme (Fe^{2+}) by the reducing milieu including glutathione. Heme (Fe^{2+}) can enter the Fenton reaction (eq 1), which participates in the protophorphyrin protein destruction due to the formation of hydroxyl radical or some higher

* To whom correspondence should be addressed. Phone: +49-6221-54-4188. Fax: +49-6221-54-5586. E-mail: elisabeth.davioud@gmx.de.
[§] Biochemie-Zentrum der Universität Heidelberg.
[†] Organisch-Chemisches Institut der Universität Heidelberg.
[#] Hygiene Institut.
[¶] London School of Hygiene and Tropical Medicine.
[⊥] Justus-Liebig-University.
[‡] Centre National de la Recherche Scientifique. E.D.-C. is delegate in the frame of a French−German cooperation with Heidelberg University, Germany.

[a] Abbreviations: CQ, chloroquine; DIEA, diisopropylethylamine; DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; ESI-MS, electrospray ionization mass spectrometry; GR, glutathione reductase; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; M5, 6-[2′-(3′-methyl)-1′,4′-naphthoquinolyl]hexanoic acid; PfCRT, *Plasmodium falciparum* chloroquine resistance transporter; TLC, thin layer chromatography; TFA, trifluoroacetic acid.

10.1021/jm7009292 CCC: $40.75
Published on Web 02/09/2008

Chart 1. Structures of 1,4-Naphthoquinones and 1,4-Dimethoxynaphthalenes and Their Related 4-Aminoquinoline Derivatives

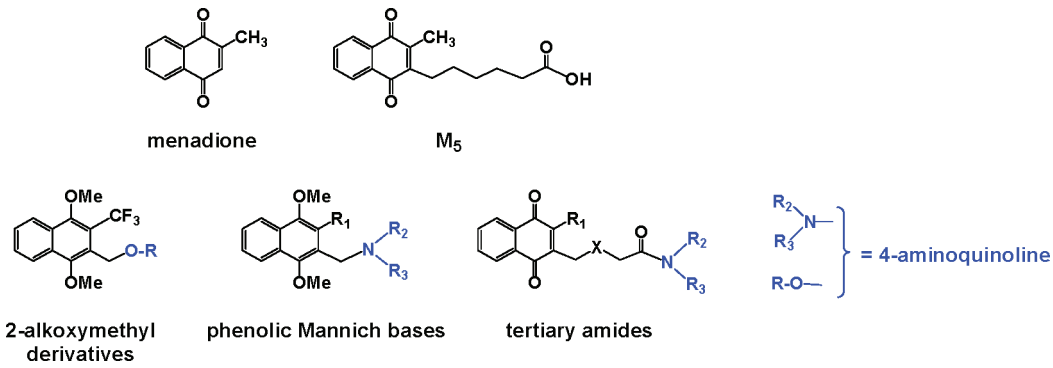
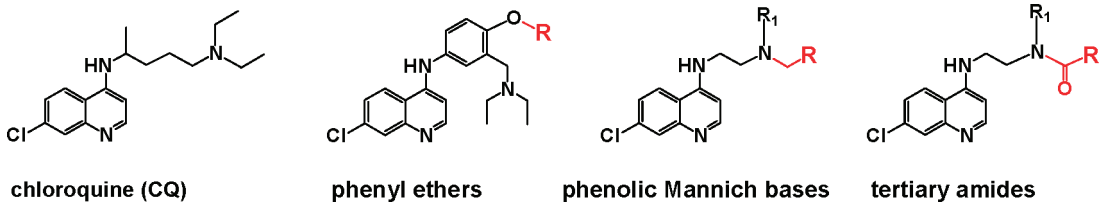


Chart 2. Structures of 4-Aminoquinoline Derivatives Based on Glutathione Reductase Inhibitors and Their Precursors



oxidation state transition metal species (eq 2), which remain bound to the heme iron and can initiate attack on the porphyrin ring.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O \quad (2)$$

This reaction is well-known in cytochrome *c* chemistry,[38] and autoxidation of hemoglobin in the presence of glutathione has been investigated at the molecular level.[39–41] Glutathione indirectly contributes to heme degradation (via the Fenton reaction) in the cytosol by redox-cycling the heme Fe^{3+} in heme Fe^{2+}.[42] The glutathione-dependent heme degradation was shown to be inhibited by 4-aminoquinolines,[43] likely by slowing the overall rate of the heme redox-cycling. This observation indeed reveals the cofacial $\pi-\pi$ sandwich-type complex formed between CQ (and the related 4-aminoquinolines) and heme Fe^{3+} in the form of two hematin μ-oxo dimers, an interaction that was exploited in numerous screening assays to measure the heme binding affinity in the presence of potential drugs.[44,45]

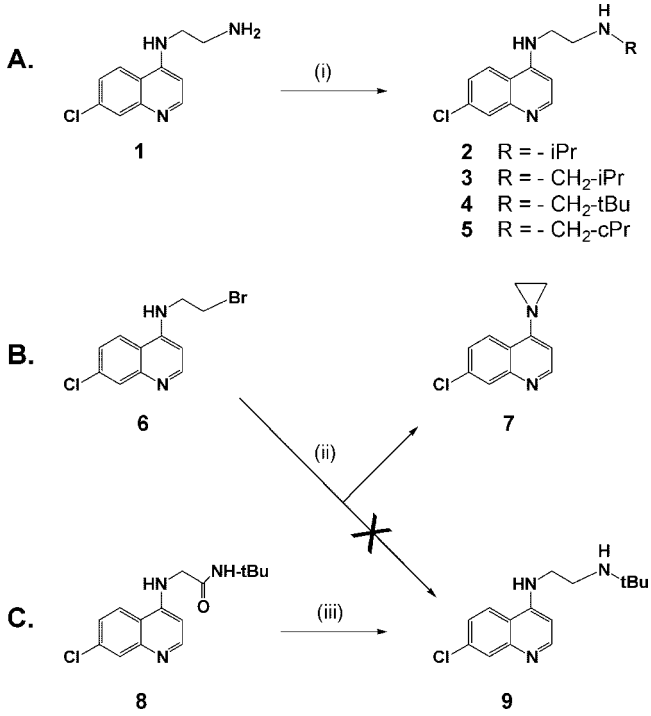
One of the most important antioxidative systems consists of (di)thiols (e.g., glutathione), which are recycled by disulfide reductases, namely, both glutathione reductases (GR) of the malarial parasite *P. falciparum* and of man and the thioredoxin reductase of *P. falciparum*.[46] An elevation of glutathione content in parasites leads to increased resistance to CQ,[47] while GSH depletion in resistant *P. falciparum* strains is expected to restore the sensitivity to CQ. An important class of inhibitors for the glutathione and thioredoxin reductases are the 1,4-naphthoquinones. Two representatives are the menadione and its hexanoic acid derivative 6-[2′-(3′-methyl)-1′,4′-naphthoquinolyl] hexanoic acid, or **M5** (Chart 1).[48,49] As previously demonstrated, they are uncompetitive inhibitors of GR with respect to both NADPH and glutathione disulfide. The first generation of double-headed prodrugs based on the GR inhibitor **M5** and 4-aminoquinolines was designed, and the compounds proved to be potent antimalarial agents both in vitro and in vivo.[48]

Alternatively the link developed between the 4-aminoquinoline and the drug was also designed in other laboratories as not being labile under physiological conditions. Such approach included the 4-aminoquinoline-based isatin derivatives,[50] the peroxide-based trioxaquine derivatives,[51,52] ferrocene−chloroquine analogues,[27,53] the 4-aminoquinolines based on inhibitors of a neutral zinc aminopeptidase,[54] or primaquine based on plasmepsin inhibitors.[55] These compounds were identified as dual drugs or Trojan horse drugs in literature.

Since 4-aminoquinolines accumulate in infected erythrocytes, we hypothesized that exploiting the heme-catalyzed oxidation reactions might provide a general and specific approach to design new 4-aminoquinolines as antimalarial agents. Oxidative N-dealkylation of amides and amines is reported in numerous drug detoxification pathways or in drug bioactivation.[56] Therefore, we designed and synthesized three series of 4-aminoquinolines (Chart 2) containing the GR inhibitors, the naphthoquinones, or their related deprotected and reduced precursors, the dimethoxynaphthalene. These motifs were linked to the basic side chain via an amide, an amine, or an ether bond (Chart 1), which might be labile under pathological conditions found in the parasite, i.e., the heme-catalyzed oxidation reactions. The 1,4-dimethoxynaphthalenes are inactive as GR inhibitors (Davioud-Charvet, unpublished data) but chemically more stable and easier to handle than the naphthoquinones. Under specific conditions found in the food vacuole of the parasites, the 1,4-dimethoxynaphthalenes might be oxidized to the active naphthoquinone species. So the function of the 4-aminoquinoline part within the dual drug is, next to its own antiparasitic activity, the accumulation and the transport of the second molecule part, the naphthoquinones, or its naphthalene precursor within the infected/parasitic cells.

For this purpose we first prepared new short CQ derivatives with alterations in the side chain to lower the molecular weight of the final dual drug while maintaining the antimalarial activity. We also prepared new 1,4-dimethoxynaphthalenes in order to generate their corresponding 1,4-naphthoquinones upon biooxidation. These new inhibitors include introduction of the trifluoromethyl group to reinforce the oxidant character of the parent menadione. Introduction of fluorine at the methyl group of menadione was recently shown to affect the redox potential values of the fluoro analogue versus menadione and to increase the GR inhibitory capacity.[57] From both components the dual

Scheme 1. Synthesis of Short Chloroquine Analogues **1**−**9**[a]



[a] Conditions: (i) (1) 1 equiv of ketone or aldehyde, 2 equiv of $Ti(O^iPr)_4$, 8 h, room temp; (2) 3 equiv of $NaBH_4$, EtOH, 15 h; (ii) ^tBu-NH_2, CsOH, 4 Å molecular sieves, DMF, 20 h, room temp; (iii) 1.05 equiv of 1 M BH_3·THF, dry THF, 30 min, 0 °C, and then 2 h, room temp.

drugs were created by linking them together in three different ways. This linkage is, in the case of the carboxylic acid $\mathbf{M_5}$ and its analogues, an amide bond and, in the other cases, an amine bridge. A few nonquinoline dual drugs were also designed with the view to establishing preliminary structure–activity relationships with respect to the role of the 1,4-dimethoxynaphthalene moiety as naphthoquinone precursor. In this case, we coupled the 1,4-dimethoxynaphthalene with two phenols known to deplete the glutathione content in the cell, paracetamol or amodiaquine, by an ether linkage. All new derivatives were tested for their antimalarial potency against two *P. falciparum* strains expressing different degrees of resistance with respect to CQ. The most active dual drugs were tested in the *Plasmodium berghei* rodent model. Additional results about the mechanism of potent antimalarial tertiary amides are presented.

Results

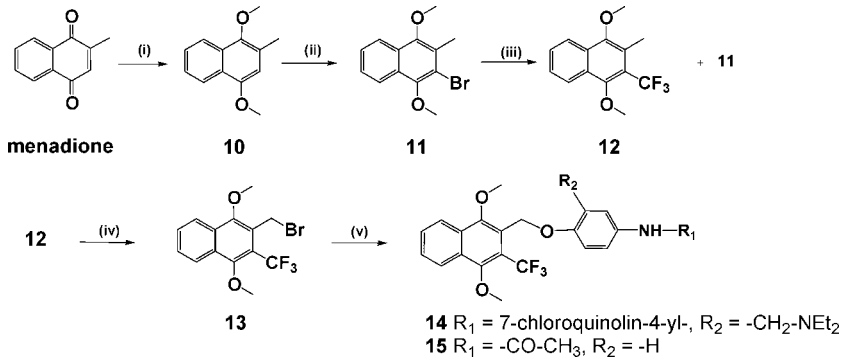
Chemistry. The short CQ analogues **2**−**5** were synthesized by reductive amination[58] of the respective carbonyl compound by *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** in the presence of $NaBH_4$ and $Ti(O^iPr)_4$ (Scheme 1A). In our hands the synthesis of the *tert*-butyl substituted 4-aminoquinoline **9** as previously described[25] did not work because the preparation of the amino side chain, the *N*-*tert*-butylethylene diamine,[59] mainly generates aziridine from 2-bromoethylamine by thermal elimination. Alternatively, another route was developed. We first attempted to substitute the bromo atom in the *N*-bromoethyl-4-aminoquinoline **6** with *tert*-butylamine in the presence of CsOH in DMF over molecular sieves[60] (Scheme 1B). But instead of the desired product, we exclusively obtained the aziridine **7** (93%). Its X-ray structure is given in the Supporting Information. The *tert*-butyl substituted 4-aminoquinoline **9** was then accessible in two steps (Scheme 1C). First, glycin-*tert*-butylamide[61] was produced according to the reported protocol developed for tryptophan.[62] Reaction of glycin-*tert*-butylamide with 4,7-dichloroquinoline giving **8** as reported was followed by BH_3·THF complex reduction to the amine **9**.

For all 1,4-dimethoxynaphthalene derivatives bearing a trifluoromethyl group the 2-bromomethyl-1,4-dimethoxy-3-trifluoromethylnaphthalene **13** was the central intermediate (Scheme 2). The bromide **13** was accessible from 1,4-dimethoxy-2-methylnaphthalene **10** through bromination at the aromatic ring with Br_2 in CH_2Cl_2 at 0 °C to the bromide **11** (86%) and then replacement of the bromine by the CF_3 group using (trifluoromethyl)copper.[63] This reagent can be produced from CF_3CO_2Na and CuI by decarboxylation in toluene/DMA at high temperatures.[64] After 8 h at 150−170 °C the 1,4-dimethoxy-2-methyl-3-trifluoromethylnaphthalene **12** was isolated in 46% yield next to 33% of the unreacted bromide **11**. It was not possible for us to increase this yield through longer reaction times, higher temperatures, or the use of DMF instead of DMA. In all cases we recovered the unreacted starting material, which was then recycled. Finally, the bromination of the methyl group in **12** with *N*-bromosuccinimide in CCl_4 and benzoyl peroxide as initiator[63,64] gave 55% of the desired 2-bromomethyl-1,4-dimethoxy-3-trifluoromethylnaphthalene **13**. For the synthesis of the dual drugs **14** and **15** (Scheme 2), amodiaquine and paracetamol were respectively deprotonated with cesium hydroxide[65] in DMF together with molecular sieves and then coupled with the bromide **13**. This procedure was superior to the use of NaH as a base. Both 2-alkoxymethyl-3-trifluoromethylhydronaphthoquinol methyl ethers **14** and **15** were produced in 82% and 93% yields, respectively.

The synthesis of the structural related compounds **19** and **20** started from the 1,4-dimethoxynaphthalenes **16** and **10** (Scheme 3A). The carbaldehyde group was first introduced by the reaction with dichloromethyl methyl ether and $SnCl_4$[66] to afford the 1,4-dimethoxynaphthalen-2-carbaldehyde **17** (92%) or the 1,4-dimethoxynaphthalen-3-methyl-2-carbaldehyde **18** (89%). Subsequent reductive amination with the 4-aminoquinoline **1** and $NaBH(OAc)_3$ in CH_2Cl_2 gave **19** (44%) or **20** (90%). The trifluoromethyldimethoxynaphthalene analogue **21** could only be prepared by substituting the bromide **13** by the terminal primary amine in the 4-aminoquinoline **1** in DMF/EtOH with an excess of NEt_3 (85%) (Scheme 3B). Its dihydrochloride salt **21** was prepared after reaction with TMSCl in methanol. The synthesis of the final tertiary amines was performed by a high-yielding reductive amination of acetone, isobutyraldehyde, or pivalaldehyde in the presence of $NaBH(OAc)_3$ as the reducing agent. Use of the secondary starting amines **19**, **20**, or **21** respectively gave the *N*-isopropyl-substituted derivatives **22** (98%), **23** (80%), or **24** (87%), the *N*-isobutyl-substituted derivatives **25** (99%), **26** (97%), or **27** (90%), and the *N*-neopentyl-substituted derivatives **28** (97%), **29** (86%), or **30** (68%). This approach was considerably more effective than the direct formation of the *N*-isopropyl- and the *N*-isobutyl-substituted derivatives by reductive amination of the carbaldehydes **17** and **18** starting from the corresponding secondary amines **2** and **3** under various conditions, e.g., $NaBH(OAc)_3$ (also under acidic conditions), $Ti(O^iPr)_4/NaBH(OAc)_3$,[67,58] $TiCl_4/NaBH_4$, or $NaBH(OAc)_3$. The Mannich base **32** was prepared from the CQ analogue **31**, 1-(7-chloroquinolin-4-yl)propane-3-isopropyl-1,3-diamine, and paracetamol through the Mannich reaction in the presence of paraformaldehyde under reflux for 3 days (Scheme 3C).

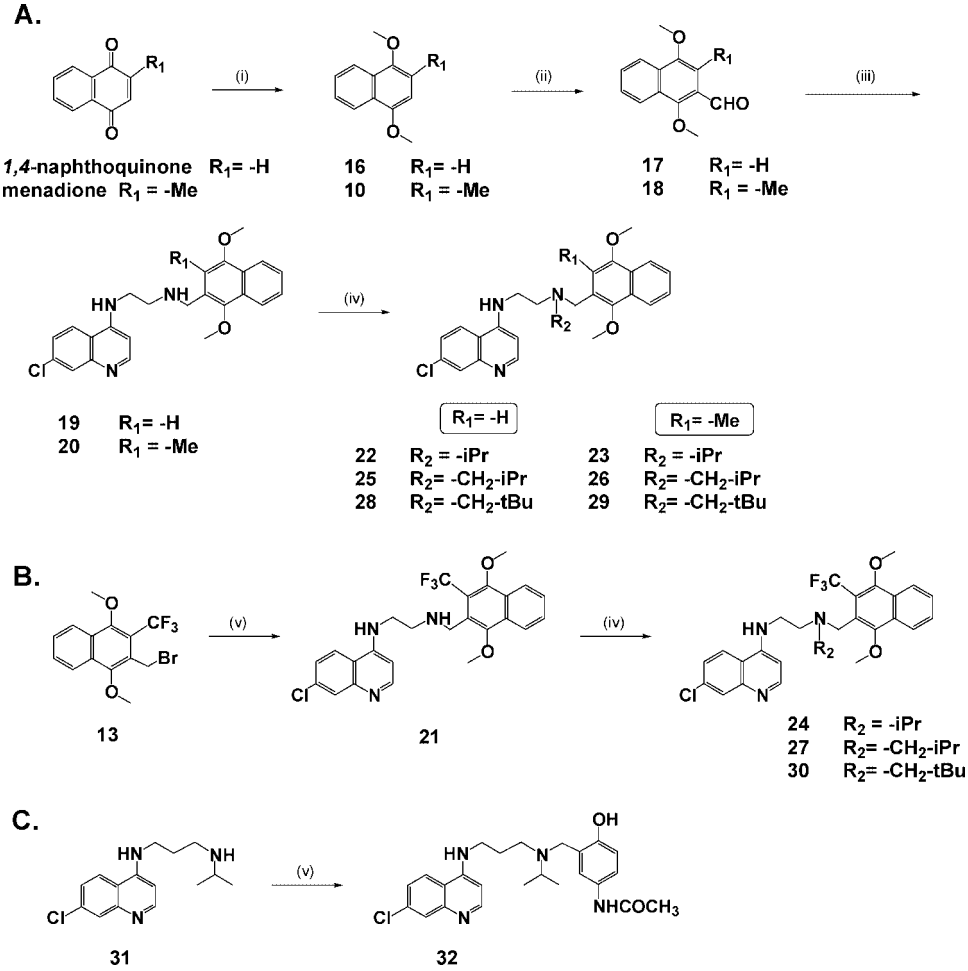
For the synthesis of the amides **34**−**36**, derived from naphthoquinones and the short CQ analogues **1** and **2** (Scheme 4), the starting acids 6-(3-methyl-1,4-dioxo-1,4-

Scheme 2. Synthesis of 2-Alkoxymethyl-3-trifluoromethylhydronaphthoquinol Methyl Ethers **14** and **15**[a]



[a] Conditions: (i) 2.5 equiv of $SnCl_2$, concentrated HCl, EtOH, 40 °C, 10 min; 2.5 equiv of KOH, 3 equiv $(CH_3O)_2SO_2$, acetone, 2.5 h, room temp; (ii) 1.05 equiv of Br_2, CH_2Cl_2, 2 h; (iii) 3 equiv of CF_3COONa, 2 equiv of CuI, DMA/toluene, 170 °C, 12 h; (iv) 1.0 equiv of NBS, benzoyl peroxide, CCl_4, reflux, 5 h; (v) (1) phenol, CsOH, 4 Å molecular sieves, DMF, 40 min, room temp at 40 °C; (2) bromide **13**, DMF, 40 °C, 6.5 h.

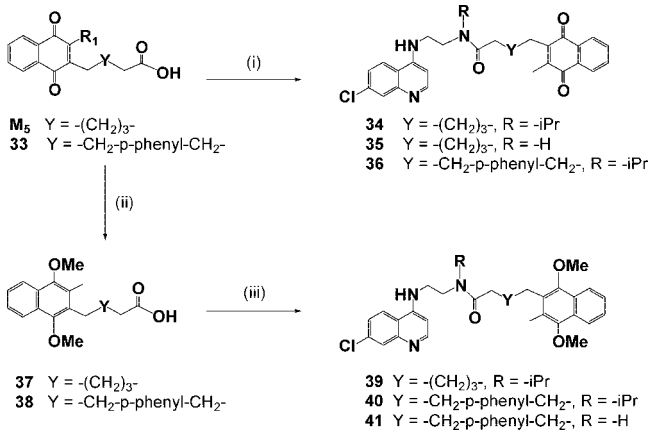
Scheme 3. Synthesis of Phenolic Mannich Bases **19–30** and **32**[a]



[a] Conditions: (i) 2.5 equiv of $SnCl_2$, concentrated HCl, EtOH, 40 °C, 10 min; 2.5 equiv of KOH, 3 equiv of $(CH_3O)_2SO_2$, acetone, room temp, 2.5 h; (ii) $SnCl_4$, $CHCl_2OCH_3$; (iii) (1) amine **1** in CH_2Cl_2, dry methanol, 2 h, room temp; (2) 3 equiv of $NaBH(OAc)_3$, 24 h, room temp; (iv) (1) excess acetone or aldehyde in CH_2Cl_2, dry methanol, 2.5 h, room temp; (2) 3 equiv of $NaBH(OAc)_3$, 48−72 h, room temp; (v) 3 equiv of amine **1** in DMF, anhydrous EtOH, 3 equiv of NEt_3, 18 h, room temp; (v) 0.5 equiv of acetamidophenol, 1.25 equiv of aqueous formaldehyde, EtOH, reflux, 79 h.

dihydronaphthalen-2-yl)hexanoic acid $\mathbf{M_5}$[68] and [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **33**[57] were activated through standard coupling conditions using HOBt/EDC (**34**, 62.1%; **35**, 19%; **36**, 23.2%). When the coupling reaction was applied in the presence of the secondary amine **2** in the presence of the starting menadione-derived carboxylic acids $\mathbf{M_5}$ and **33**, low to moderate yields of the desired compound were obtained because of the formation of a mixture consisting of two main products. The mixture was separated by several fractionations through silica gel chromatography columns. The main product (highest R_f, yellow powder after desiccation) was the desired tertiary amide. The second product (lowest R_f, brown gum after desiccation) was assigned as a polymer formed upon proton abstraction at the methyl group of the menadione moiety by the basic secondary amine **2** (addition to the quinone

Scheme 4. Synthesis of Tertiary Amides **34–36** and **39–41** Based on $\mathbf{M_5}$, Its Benzyl Analogue **33**, and Their Deprotected Reduced Precursors **37** and **38**[a]



[a] Conditions: (i) 1 equiv of HOBt, 1.3 equiv of EDC, 1.5 equiv of amine, DMF, overnight, room temp; (ii) (1) 3.7 equiv of $SnCl_2$, concentrated HCl, EtOH, 40 °C, 2.5 h; (2) 4.4 equiv of $(CH_3O)_2SO_2$, acetone, 5.4 equiv of KOH dropwise, 60 °C, 3.5 h; (iii) (1) 5.0 equiv of $SOCl_2$; (2) 1.2 equiv of amine, NEt_3, CH_2Cl_2, 1 h, 0 °C, then overnight, room temp.

methide). The corresponding 1,4-dimethoxynaphthalene derivatives **39**−**41** were also prepared as potential dual drugs. 6-(1,4-Dimethoxy-3-methylnaphthalen-2-yl)hexanoic acid **37** and [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38** were first transformed into their respective acid chloride with thionyl chloride in CH_2Cl_2 and then coupled in a one-pot reaction to the appropriate 4-aminoquinoline **1** or **2**. The yields of the coupling reaction were low to moderate **39** (7%), **40** (61.4%), and **41** (14.6%). The tertiary amide **36** was alternatively prepared by oxidation of **40** with cerium ammonium nitrate (CAN) in acetonitrile/water in 62% yield.

Inhibition Assay of *β*-Hematin Formation. Four representative compounds, CQ, the short CQ analogue **2**, and the tertiary amide **34**, were tested for their ability to inhibit the polymerization of hematin in vitro using the colorimetric *β*-hematin inhibition screening assay[45] with slight modifications described in the experimental procedure. We selected DMF as the solvent because the tertiary amide **34** precipitated in other solvents (aqueous HCl and MeOH or aqueous HCl and *n*-butanol). CQ was used as reference inhibitor of *β*-hematin formation. The GR inhibitor $\mathbf{M_5}$ was also tested to evaluate separately the interference of the non-quinoline portion of the dual drug **34** with the formation of *β*-hematin As controls, we also measured (i) the formation of *β*-hematin in the absence of the drug with or without DMF (0% polymerization) and (ii) the hematin content without preincubation for 1 h at 60 °C with or without DMF (100% polymerization). The results of the experiment were expressed as IC_{50} values representing the molar equivalents of tested compounds, relative to 1 equiv of hematin, required to inhibit the *β*-hematin formation by 50% (reproducible data from three experiments). Considering the dose-response curves of CQ and the short CQ analogue **2**, the absorbance first decreased from 0 to 1 for CQ (red curve, Figure 1), and from 0 to 2 for **2** (green curve, Figure 1), revealing the shift of the equilibrium between the dimer H_2O/HO-Fe(III)PPIX in aqueous solution[69] to the *μ*-oxo dimer. This shift is accompanied by the release of free sites of hematin because water bound to HO-Fe(III)PPIX likely prevents the binding of pyridine in accordance with the A405 decrease as the drug

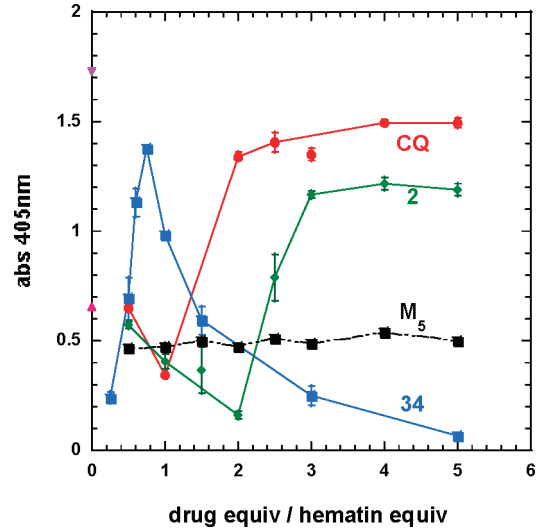
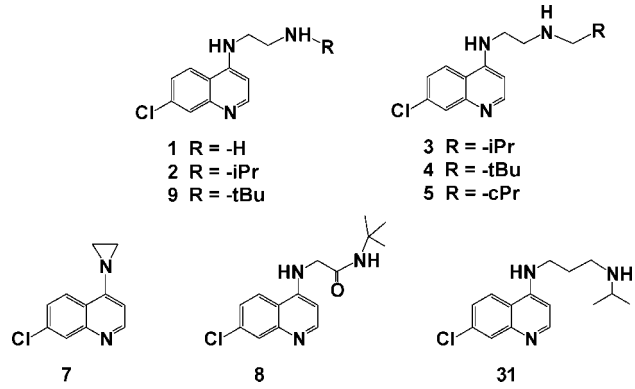


Figure 1. Inhibition of *β*-hematin formation by the 4-aminoquinolines, chloroquine, the short chloroquine analogue **2**, the tertiary amide **34**, and the glutathione reductase inhibitor $\mathbf{M_5}$. IC_{50} values for *β*-hematin inhibition were determined from the absorbance at 405 nm versus drug (equiv)/hematin (equiv). The solid line is a dose-response curve resulting from interpolated experimental data points measured in duplicate for CQ (red circle), the short CQ analogue **2** (green circle), the tertiary amide **34** (blue square), the GR inhibitor $\mathbf{M_5}$ (black square). As controls, (i) the formation of *β*-hematin in the absence of the drug (red triangle, 100% polymerization) and (ii) the hematin content without preincubation for 1 h at 60 °C (blue triangle), 0% polymerization) are shown on the *Y*-axis.

equivalent number increased from 0. Then, when the drug has saturated all the sites of the *μ*-oxo dimer, the inhibition of the hematin polymerization reached the maximum level in agreement with the A405 value to the highest point. Under the conditions described, CQ showed an IC_{50} value of 1.6 and the maximal inhibition was reached at a drug/hematin ratio of ∼2:1; these values are similar to the reported values.[45] The short CQ analogue **2** and the tertiary amide **34** displayed IC_{50} values of 2.6 and 0.5 with a drug/hematin ratio of ∼3:1 and 0.9:1, respectively, while the GR inhibitor $\mathbf{M_5}$ displayed no inhibition of the *β*-hematin formation. Thus, the tertiary amide **34** displayed a potent *β*-hematin inhibitory activity when compared with CQ and the short CQ analogue **2** and with the non-quinoline portion $\mathbf{M_5}$ (Figure 1), suggesting that both components of the tertiary amide are involved in the hematin interaction.

Antimalarial Activity and Cytotoxicity of the Compounds in Vitro. All compounds synthesized were tested for their antiparasitic activity on the CQ-sensitive *P. falciparum* strain 3D7 and the CQ-resistant strain K1. In parallel, cytotoxicity of the compounds was determined on human KB cells. The results are expressed as IC_{50} and TD_{50} values representing the drug concentration required to inhibit the growth of parasites and human cells, respectively, by 50%. The ratio (IC_{50} of K1)/(IC_{50} of 3D7) was also determined and is known to increase with the cross-resistance toward chloroquine. Data for the short CQ analogues are summarized in Table 1, for dual drugs based on amodiaquine and paracetamol in Table 2, for the phenolic Mannich bases in Table 3, and for the tertiary amides in Table 4. Among the short CQ analogues (Table 1) the *N*-isobutyl- and the *N*-neopentyl-substituted derivatives **3** and **4** exhibited the most potent antimalarial effects with the highest activity for **4** against the CQ-resistant strain K1. The excellent activity of both compounds is consistent with the activity of short CQ analogues containing

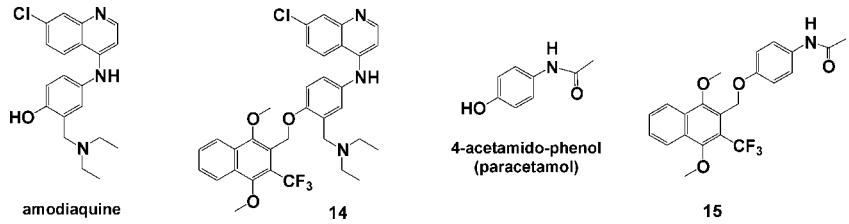
Table 1. Antimalarial Activity of Short Chloroquine Analogues **1–9** and **31**



compd	R	IC_{50} 3D7[a] (nM)	IC_{50} K1[a] (nM)	(IC_{50} K1)/(IC_{50} 3D7)[b]	TD_{50} KB[c] (μM)
1	−H	4.5	1800	400	49.6
2	$-{}^{i}$Pr	10.2	96.7	9.5	89.8
3	$-CH_2-{}^{i}$Pr	22.8	72.0	3.1	22.5
4	$-CH_2-{}^{t}$Bu	20.6	6.8	0.3	62.1
5	$-CH_2-{}^{c}$Pr	2.7	145.0	53.7	71.9
7		16000	39235	2.4	101.6
8		993.9	1,439	1.4	194.0
9	$-{}^{t}$Bu	21.2	127.8	6.0	142.9
31		3.6	144.0	40	47.9
CQ		15.0	519.3	18.7	127.33

[a] In these assays, 3D7 and K1 are CQ-sensitive and -resistant *Plasmodium falciparum* strains, respectively. The standard drugs chloroquine (CQ) and amodiaquine (AQ) served as positive controls for CQ-sensitive *P. falciparum* 3D7 strain and CQ-resistant *P. falciparum* K1 strain, respectively. [b] The ratio increases with the cross-resistance toward CQ. [c] The cytotoxicity is evaluated against KB cells. Podophyllotoxin exhibited an IC_{50} value of 0.07 μM against the human KB cell line.

Table 2. Antimalarial Activity of Potential Dual Drugs **14** and **15** Based on Amodiaquine and Paracetamol

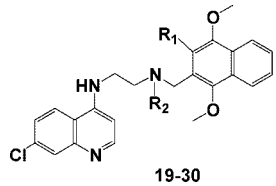


compd	type	IC_{50} 3D7[a]	IC_{50} K1[a]	(IC_{50} K1)/(IC_{50} 3D7)[b]	TD_{50} KB[c] (μM)
14	dual drug	32.0	32.0	1.0	15.0
15	dual drug	6438	15737	2.4	>715
AQ	reference	9.7	6.4	0.6	nd
paracetamol	reference	>198400	86700	<0.4	nd

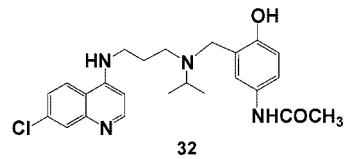
[a] In these assays, 3D7 and K1 are CQ-sensitive and -resistant *Plasmodium falciparum* strains, respectively. The standard drug amodiaquine (AQ) served as positive control for chloroquine-sensitive *P. falciparum* 3D7 strain and chloroquine-resistant *P. falciparum* K1 strain. [b] The ratio increases with the cross-resistance toward CQ. [c] The cytotoxicity is evaluated against KB cells. Podophyllotoxin exhibited an IC_{50} value of 0.07 μM against the human KB cell line.

an ethylenediamine-based side chain and an increased antimalarial activity against the CQ-resistant strain K1.[25,26] The design of the dual drugs built in this study from both phenols, amodiaquine and paracetamol, and the 2-alkoxymethyl-3-trifluoromethyl-1,4-dimethoxynaphthalene did not afford improved antimalarial effects with respect to the parent phenols (Table 2). The data of the second series of dual drugs based on phenolic Mannich bases (Table 3) revealed significant antimalarial effects, depending on the substitution both of the *N*-alkyl (R_2) side chain of the 4-aminoquinoline and the substitution of the 1,4-dimethoxynaphthalene (R_1). The most potent antimalarial dual drugs are the 4-aminoquinolines with an unbranched side chain (R_2 = H), but they are also the most cytotoxic ones against the human cells. Noteworthy is the fact that they are more active than the parent short CQ analogue 1. Among these three derivatives the most active and the less toxic is the derivative **21** built from the 3-trifluoromethyl-1,4-dimethoxynaphthalene. In each subseries of 1,4-dimethoxynaphthalenes (R_1 = H, Me, CF_3) the cytotoxicity decreased with the branching of the *N*-alkyl substituent (R_2 = ^{i}Pr) of the 4-aminoquinoline but also coincided with a decrease of the antiplasmodial activity. When the branching of the alkyl chain was moved from the N atom by adding a methylene group, both the antimalarial effects and the cytotoxicity increased suggesting a common origin to both effects, likely through the generation of a quinone methide upon oxidation followed by Michael addition (data not shown). Noteworthy is that oxidation of the final phenolic Mannich bases by CAN resulted in a rapid degradation of the final naphthoquinone upon elimination of the amine moiety. For this reason the phenolic Mannich bases under their 1,4-dimethoxynaphthalene forms were tested as prodrugs (precursors) of naphthoquinones against the parasites in cultures. Finally tertiary amides were prepared starting from the short CQ analogues **1** and **2** and the carboxylic acids $\mathbf{M_5}$ and **33**, identified earlier as potent GR inhibitors, or their

Table 3. Antimalarial Activity of Phenolic Mannich Bases **19–30** and **32**



19-30

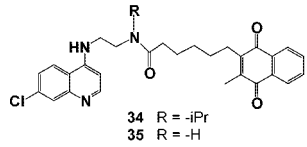


32

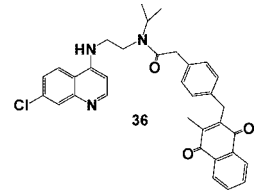
compd	R_1	R_2	IC_{50} 3D7[a] (nM)	IC_{50} K1[a] (nM)	TD_{50} KB[b] (μM)
CQ			12.3	596.7	127.3
19	−H	H	23.7	28.4	3.8
22	−H	^{i}Pr	517.2	344.8	67.0
25	−H	CH_2-^{i}Pr	146.4	188.3	43.8
28	−H	CH_2-^{t}Bu	237.2	154.1	8.8
20	−Me	H	229.4	22.9	5.1
23	−Me	^{i}Pr	83.7	104.6	85.8
26	−Me	CH_2-^{i}Pr	182.9	40.6	30.6
29	−Me	CH_2-^{t}Bu	155.7	134.2	10.6
21	$-CF_3$	H	127.5	16.0	28.5
24	$-CF_3$	^{i}Pr	183.8	298.9	35.4
27	$-CF_3$	CH_2-^{i}Pr	162.1	198.3	11.9
30	$-CF_3$	CH_2-^{t}Bu	146.0	115.7	12.0
32			22.7	317.5	180.5

[a] In these assays, 3D7 and K1 are CQ-sensitive and -resistant *Plasmodium falciparum* strains, respectively. The standard drug chloroquine (CQ) served as positive control for chloroquine-sensitive *P. falciparum* 3D7 strain and chloroquine-resistant *P. falciparum* K1 strain, respectively. [b] The cytotoxicity is evaluated against KB cells. Podophyllotoxin exhibited an IC_{50} value of 0.07 μM against the human KB cell line.

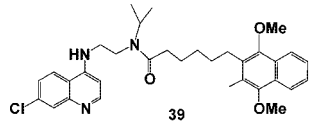
Table 4. Antimalarial Activity of Tertiary Amides **34–36** and **39–41** Based on $\mathbf{M_5}$, Its Benzyl Analogue **33**, and Their Deprotected Reduced Precursors **37** and **38**



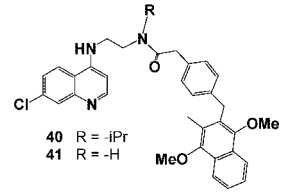
34 R = -iPr
35 R = -H



36



39



40 R = -iPr
41 R = -H

compd	type	IC_{50} 3D7[a] (nM)	IC_{50} K1[a] (nM)	(IC_{50} K1)/(IC_{50} 3D7)[b]	TD_{50} KB[c] (μM)
1	CQ analogue	4.5	1,800	400	49.6
2	CQ analogue	10.2	96.7	9.5	89.8
34	dual drug	7.5	9.4	1.1	37.0
35	dual drug	183.9	367.3	2.0	22.2
36	dual drug	12.4	5.3	0.4	318.32
39	dual drug	52.8	302.4	5.7	6.9
40	dual drug	318.7	268.3	0.8	1.7
41	dual drug	288.8	505.3	1.7	11.51
CQ	reference	5.8 −15.0	571.0	18.7	127.33

[a] In these assays, 3D7 and K1 are CQ-sensitive and -resistant *Plasmodium falciparum* strains, respectively. The standard drug chloroquine (CQ) served as positive control for CQ-sensitive *P. falciparum* 3D7 strain and CQ-resistant *P. falciparum* K1 strain. [b] The ratio increases with the cross-resistance toward CQ. [c] The cytotoxicity is evaluated against KB cells. Podophyllotoxin exhibited an IC_{50} value of 0.07 μM against the human KB cell line.

reduced and deprotected forms. Both tertiary amides **34** and **36** showed a higher activity than the parent 4-aminoquinoline **2** and the parent 1,4-naphthoquinones to equally kill sensitive and resistant parasites. Because these tertiary amides do not possess weak base properties as CQ and its short analogues, another mechanism of action is strongly suggested to be involved in their antimalarial activity.

In Vivo Antimalarial Activity Against *P. berghei* in Mice. Three of the most active compounds (**4**, **21**, **34**), representative of each class, were tested in *P. berghei* infected mice by intraperitoneal administration. Results of in vivo screens for the three compounds conducted against chloroquine-sensitive *P. berghei* ANKA BALB/c mice according to the Peters's 4-day test[70] are given in Table 5. For comparative purposes, data acquired in the same screens for CQ and the three derivatives are included. The most active drug **4**, the short CQ analogue with a side chain containing a *N*-neopentylamine group, caused a 94% reduction in parasitemia at 30 mg/kg with no overt toxicity. Both dual drugs **21** and **34**, based on 1,4-dimethoxy-2-trifluoromethylnaphthalene and $\mathbf{M_5}$, respectively, showed moderate activity (18% reduction in parasitemia) on a 4-day treatment with a daily dose of 21

Table 5. In Vivo Antimalarial Response of *P. berghei* Infected Mice Treated with 4-Aminoquinolines **4**, **21**, and **34**

	control untreated	CQ, 10 mg/kg po, ×4	**4**,[a] 30 mg/kg ip, ×4	**21**,[a] 30 mg/kg ip, ×4	**34**, 21 mg/kg ip, ×4
mean % parasitemia	15.28	0.13	0.86	12.48	12.50
95% clearance	1.64	0.10	0.31	2.82	2.65
standard deviation	1.18	0.08	0.35	3.21	3.03
% inhibition	0	99.2	94.4	18.3	18.2

[a] The chlorohydrate salt was used.

Table 6. Drug Combination Assays Using Newly Synthesized 4-Aminoquinolines and Clinically Used Antimalarials[a]

drug	**4**	**14**	**20**	**34**	**36**	**40**
CQ	A	nd	nd	A	nd	A
1:3	1.1			1.1		1.1
1:1	1.2			1.2		1.1
3:1	1.2			1.1		1.1
methylene blue	A	A	A	A	A	A
1:3	1.3	1.3	1.3	1.0	1.1	1.3
1:1	1.3	1.7	1.4	1.3	1.1	1.3
3:1	1.3	2.2	1.6	1.3	1.2	1.2
mefloquine	A	A	A	nd	A	nd
1:3	1.2	1.4	1.4		1.1	
1:1	1.2	2.1	1.8		1.1	
3:1	1.1	2.1	1.7		1.0	
amodiaquine	D	A	A	nd	nd	nd
1:3	1.2	1.2	1.1			
1:1	1.0	1.3	1.2			
3:1	1.0	1.3	1.3			
piperaquine	nd	nd	A	nd	nd	nd
1:3			1.2			
1:1			1.2			
3:1			1.4			
artemisinin	A		A	S	S	A
1:3	1.3	nd	1.4	0.9	0.8	1.2
1:1	1.3		1.3	0.8	0.7	1.3
3:1	1.2		1.5	0.9	0.8	1.4
artemether	A	A	A	nd	nd	nd
1:3	1.3	1.5	1.3			
1:1	1.3	1.6	1.5			
3:1	1.2	2.0	1.5			
artesunate	A	A	A	nd	nd	nd
1:3	1.3	1.1	1.1			
1:1	1.7	1.5	1.5			
3:1	1.5	2.2	1.7			

[a] In columns 2–7 FIC_{50} values are given that were obtained at fixed ratio drug combinations listed in column 1. FIC_{50} values were determined according to the reported method.[90] $FIC_{50} < 1$ indicates synergistic drug action. $FIC_{50} = 1$ indicates additive action. $FIC_{50} > 1$ indicates antagonistic action. $FIC_{50}(A) = [IC_{50}(A+B)]/IC_{50}(A)$. $FIC_{50}(B) = [IC_{50}(B+A)]/IC_{50}(B)$. $FIC_{50} = FIC_{50}(A) + FIC_{50}(B)$. IC_{50} values determined for the clinically used antimalarials (monotherapy) in the experiments were as follows: CQ, 155 ± 11.4 nM; methylene blue, 5.4 ± 0.6 nM; mefloquine, 4.85 ± 1.0 nM; amodiaquine, 9.5 ± 1.2 nM; piperaquine, 14.6 ± 2.2 nM; artemisinin, 4.5 ± 0.7 nM; artemether, 2.8 ± 0.5 nM; artesunate, 2.3 ± 0.4 nM.

mg/kg (**34**) or 30 mg/kg (**21**). This might partially result from the poor bioavailability of the compound and the low solubility in organic and aqueous solvents.

Drug Combination Assays with Clinically Used Antimalarials. Because of the increasing resistance of *Plasmodium* to presently available drugs, the development of efficient drug combination therapies is currently favored. To test the potential of the newly developed GR inhibitors as components of combination therapies, we carried out drug combination assays with chloroquine, methylene blue, mefloquine, amodiaquine, piperaquine, artemisinin, artemether, and artesunate. For these assays we used at least one compound with good antimalarial activity from each class, namely, **4**, **14**, **20**, **34**, **36**, and **40** (Tables 1–4). The results are summarized in Table 6. The short CQ analogue **4** showed slight antagonistic action in combination with basically all drugs tested. Only for amodiaquine was a tendency toward additive action observed. Most of the dual drugs tested were found to act mainly antagonistically in the drug combinations tested. The exceptions were **34** and **36**, which showed a slight synergy with artemisinin. Interestingly, both compounds **34** and **36** are tertiary amides with related structures. These structure–activity relationships are promising, and the mechanisms of action of these tertiary amides are being studied in further detail and will be reported elsewhere. It should be mentioned, however, that besides a promising synergistic action in cell culture, the success of a drug combination therapy is largely defined by absorption, distribution, metabolization, and excretion, defining, for example, the half-life of a compound in vivo.

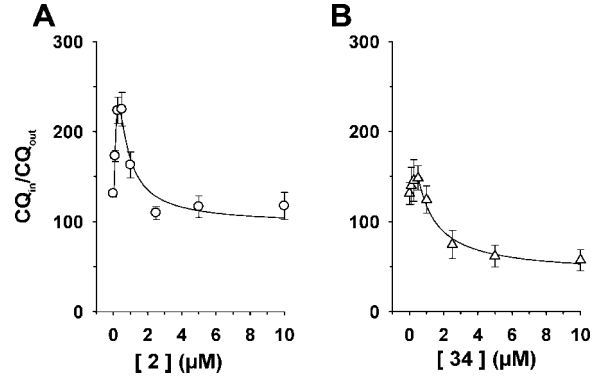


Figure 2. Effect of preloaded 4-aminoquinolines **2** (A) and **34** (B) on labeled CQ accumulation by erythrocytes infected with the CQ-resistant *P. falciparum* parasite Dd2. The level of labeled CQ accumulation, given as the ratio of the intracellular vs extracellular CQ concentration (CQ_{in}/CQ_{out}), is analyzed as a function of the extracellular concentration of **2** and **34** used for preloading. The data points were fitted using previously described equations,[18] which assume as carrier-model for **2** or a simple binding model for **34**. The mean (SEM) of four or more independent determinations is shown.

Competition Studies on Chloroquine Transport. Previous studies have demonstrated trans-stimulated chloroquine movement in CQ-resistant parasites;[18,19,22] i.e., CQ present on the trans face of the membrane was able to stimulate CQ movement from the cis to the trans site. This phenomenon was linked to the PfCRT polymorphisms associated with CQ resistance.[21,22,71] These data have led to the hypothesis that CQ resistance is based on the acquisition of a PfCRT-mediated CQ efflux system.[21,22] In order to determine whether the short CQ analogue **2** and the tertiary amide **34**, which has no basic function at the side chain, interfere with the putative CQ transport, the compounds were investigated in a trans-stimulation assay[19] in which preloaded **2** and **34** were used to stimulate labeled CQ uptake into the CQ-resistant *P. falciparum* parasite Dd2 (Figure 2). To this end, erythrocytes infected with trophozoites of Dd2 were preloaded with different concentrations of **2** or **34**, ranging from 0 to 10 μM, for 15 min before the cells were washed and placed in medium containing 43 nM [^{3}H]CQ. The cellular CQ accumulation ratio (intracellular versus extracellular CQ concentration) was determined and analyzed as a function of the preloaded **2** or **34** concentration. In the case of **2**, the CQ analogue with a short aminoalkyl side chain, the curve first rises and then falls with increasing preloaded **2**, consistent with trans stimulation

of labeled CQ uptake by **2**. In the case of the tertiary amide **34** no such trans stimulation was observed. The curve falls with increasing preloaded concentrations of **34**. This behavior is distinct from that seen with **2** and suggests a simple binding process in which both **34** and CQ compete for binding to common sites, possibly heme.

Discussion

Our strategy is aimed at the design of antimalarial dual drugs based on a short CQ analogue attached to a GR inhibitor or a glutathione depletor. Our previous studies with GR enzymes revealed two types of mechanism-based inhibitors: the reversible uncompetitive inhibitors like the carboxylic acids $\mathbf{M_5}$ and **33**; the subversive substrates like menadione and the 1,4-naphthoquinone. A more recent work with a fluoromethyl analogue of $\mathbf{M_5}$ showed the influence of the fluorine atom at the methyl group of menadione on the redox potential value of the final molecule, resulting in an increased oxidant character. Thus, in the present work, we produced dual drugs based on the two types of inhibitors: (i) the carboxylic acids and (ii) the 1,4-naphthoquinone, menadione, and the trifluoromenadione or their reduced dimethoxynaphthalene forms. For glutathione depletors, we used paracetamol and amodiaquine as starting phenols.

First, we focused on the preparation of short CQ analogues (Scheme 1) that retain the activity of CQ and are not too bulky to allow the chemical attachment of the redox active moiety. Among the short CQ analogues, the *N*-neopentyl-substituted 4-aminoquinoline **4** was found to be the most active both in vitro and in vivo. However, we selected the less bulky *N*-isopropyl analogue **2** instead of the *N*-^{t}Bu- or the *N*-CH_2-^{t}Bu because of (i) its effective preparation via reductive amination of acetone, (ii) the lower hindrance observed in the reactions involving the secondary amine function of the side chain, (iii) its antimalarial activity similar to that of CQ, and (iv) the low molecular weight (MW) allowing the preparation of dual drugs with a final MW approaching 500. From the selected short CQ analogue **2**, we synthesized two effective series of dual drugs, the phenolic Mannich bases (Scheme 3) and the tertiary amides (Scheme 4). All phenolic Mannich bases (Table 3) showed significant antimalarial activities in the nanomolar range. The N-unsubstituted 4-aminoquinolines ($R_2 = H$, **19**–**21**) gave potent dual drugs with the lowest IC_{50} values in the nanomolar range but also the lowest TD_{50} values against human cells. In order to limit the cytotoxicity, we synthesized the *N*-alkyl analogues ($R_2 = {}^i$Pr, **22**–**24**) resulting in lower cytotoxicity but also lower antimalarial activities. When the branched alkyl chain was extended with one methylene group at the N atom ($R_2 = CH_2$-^{i}Pr, CH_2-^{t}Bu), both the antimalarial and the cytotoxic effects increased again. In this series of dual drugs we were unable to dissociate these two effects suggesting a common mechanism likely involving the formation of a quinone methide.

The second series of dual drugs is illustrated with the amides (Table 4) built from the short CQ analogues **1** and **2** and the carboxylic acids or their reduced and deprotected forms. The most effective were the tertiary amides **34** and **36** with excellent antimalarial activities against CQ-sensitive and -resistant parasites in the very low nanomolar range (3D7, 7−12 nM; K1, 5−10 nM). Some tertiary amides in the side chain of 4-aminoquinolines were also reported to display significant antimalarial activities.[72,73] In the case of the tertiary amide **34**, in comparison to the parent short CQ analogue **2**, we examined its ability to compete for the same intraparasitic receptor of CQ, the (Fe^{III})protoporphyrin, and for the CQ transporter. In the hematin polymerization test, as expected, the short CQ analogue **2** showed an activity similar to CQ to inhibit the formation of β-hematin in vitro. Thus, the antimalarial activity of **2** (3D7, ∼10 nM; K1, ∼100 nM), in the same range as CQ, can correlate with the β-hematin inhibitory activity. However, under the same conditions, the tertiary amide **34** was found to inhibit the β-hematin formation at very low equivalent number versus hematin. In addition, the behavior of this compound in this test, in accordance with the curve shapes, by comparison to CQ and its analogues, strongly suggests a mode of action distinct from the CQ mode of action. Noteworthy is the fact that the antimalarial activity of **2** and **34** was in the nanomolar range despite the loss of the basic character at the terminal side chain of **34**. These results proved that the terminal tertiary nitrogen is not essential to kill resistant parasites and might be associated with drug resistance.

The different behavior of the short CQ analogue **2** and the tertiary amide **34** in a CQ trans-stimulation assay is consistent with this hypothesis. Since polymorphisms within PfCRT have been linked with altered responses to a range of structurally and functionally distinct antimalarial drugs,[9−11,74] we wondered whether PfCRT can act on **2** and **34**. By use of the established trans-stimulation protocol,[18] the short CQ analogue **2** revealed rather complex kinetics. The stimulated CQ uptake at low concentration of preloaded **2** to levels above the zero-trans control (cells not preloaded with **2**) suggests the presence of a carrier-mediated transport process and can be interpreted as follows: the preloaded **2** competes with incoming labeled CQ for efflux by the same carrier, presumably PfCRT.[18] Only at high concentrations of preloaded **2**, when the carrier is fully saturated, does the uptake curve fall, possibly because of both **2** and CQ competing for binding to heme. It is worth mentioning that **2** is a derivative of CQ with a shortened aminoalkyl side chain. Thus, a mode of action for short CQ analogues similar to that of CQ leading to cross-resistance with CQ is possible. Cross-resistance with CQ has been described for a number of side chain variants of CQ.[23,24] In the case of **34**, no evidence for stimulated CQ uptake was found. The decline of the uptake curve with increasing amounts of intracellularly preloaded **34** is consistent with a model in which incoming labeled CQ competes with preloaded **34** for common binding sites, possibly heme. Alternatively, the tertiary amide **34** may affect the pH in the parasite's digestive vacuole, which in turn would reduce acidotropic partitioning of the amphiphilic diprotic weak base CQ in this organelle. Consequently no cross-resistance with CQ is expected for the tertiary amide **34** in accordance with high antimalarial effects against the CQ-resistant K1 strain.

In the present study, we designed various series of dual drugs built from a short CQ analogue and a GR inhibitor (or a precursor) linked together via different linkages. In the absence of evidence for amide/amine cleavage in vivo, mechanisms by which tertiary amines or tertiary amides exert their antimalarial action remain elusive. Possibly, after cleavage, the simultaneous inhibition of GR and one (several) target(s) may result in potentiation of the antimalarial activity by interfering with the redox equilibrium and parasite development. In this context, cleavage of a tertiary amide bond under specific biomimetic conditions found in the food vacuole of the parasites mimicking the $cytP_{450}$-catalyzed oxidation reactions might be possible.[75] Hydrolysis of tertiary amides is also known upon complexation with copper(II) ions through a Lewis acid effect.[76,77] Different members of the protease families involved in hemoglobin degradation could be candidates for the proteolysis of the tertiary amide bond of **34** in *P. falciparum*. Thus, the antimalarial action of the newly synthesized tertiary amides deserves to be discussed

with cautious considerations until a complete study is done in the future. Further work is aimed at the evaluation of the reactivity of the tertiary amide **34**, its rate of hydrolysis under both chemical and biological conditions, and/or its complexation properties with the biologically relevant iron(II) or iron(III) species.

When tested for in vivo efficacy in a *P. berghei* infected mouse model, the 4-aminoquinolines failed to cure the infection at 30 mg/kg given intraperitoneally. No toxicity in infected mice was observed. While compound **4** is similar to other short CQ analogues, it might be considered as a dual drug because after oxidative N-dealkylation it is expected to release trimethylacetaldehyde. Trimethylacetaldehyde was reported to be deformylated at high rate by cytP_{450} isoforms and to release isobutylene,[78] which could elicit metabolic activation to become potentially harmful[79] for the parasite.

Taken together, our strategy can serve for the rational design of more potent and less toxic antimalarial dual agents after the link between both entities (a 4-aminoquinoline and a GR inhibitor) is optimized. Furthermore, the drug combination studies showed an interesting trend with the synergistic action of the tertiary amides with artemisinin that deserves to be exploited in the future drug development against malaria.

Experimental Section

Chemistry. Chemicals. The starting compounds *N*′-[7-chloroquinolin-4-yl)ethane-1,2-diamine **1**,[80] 4-(2-bromoethylamino)-7-chloroquinoline **6**,[81] *N*-*tert*-butyl-2-(quinolin-4-ylamino)acetamide **8**,[82] 1,4-dimethoxy-2-methylnaphthalene **10**,[63] 2-bromo-3-methyl-1,4-dimethoxynaphthalene **11**,[83] 1,4-dimethoxynaphthalene **16**,[84] 1,4-dimethoxynaphthalene-2-carboxaldehyde **17**,[66] 1,4-dimethoxynaphthalene-3-methyl-2-naphthalenecarboxaldehyde **18**,[85] *N*-(7-chloroquinolin-4-yl)propane-3-isopropyl-1,3-diamine **31**,[1] and 4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylmethyl)phenyl]acetic acid **33**[57] were synthesized according to reported procedures. *N*′-(7-Chloroquinolin-4-yl)-*N*-isopropylethane-1,2-diamine **2**,[1] 4-(1-aziridinyl)-7-chloroquinoline **7**,[60] and *N*′-(7-chloroquinolin-4-yl)-*N*-*tert*-butylethane-1,2-diamine **9**[25] are known compounds but were produced according to new procedures. All commercially available chemicals were purchased from Acros and Aldrich and used without further purification. All solvents for the reaction were dried before use (solvents in p.a. quality). CH_2Cl_2 was distilled under nitrogen atmosphere from $CaCl_2$. The used petroleum ether had a bp of 40–60 °C.

General Methods. New derivatives were isolated by silica gel chromatography (silica gel 60, Merck 230–400 mesh) of the crude reaction mixture. Thin-layer chromatography (TLC) was carried out on flexible Machery & Nagel Polygram SIL G/UV-254 and Alugram SIL G/UV-254 silica gel sheets (0.2 mm). The components were detected by their absorption at 254 nm, followed by staining with 10% ninhydrine in acetone and heating. The purity of the isolated compounds was checked by elemental analysis. Elemental analyses were performed on an Elementar vario EL at Organisch-Chemisches Institut, University of Heidelberg. Electrospray ionization mass spectra (ESI-MS) of the compounds were recorded by using a Finnigan-MAT (San Jose, CA) model TSQ 7000 at the Biochemie-Zentrum of Heidelberg University. Melting points were determined on a Büchi melting point apparatus and were not corrected. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker AC 300 and DRX-300 MHz spectrometers, and the chemical shifts were expressed in ppm relative to TMS. Multiplicity was indicated as s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), and m (multiplet). The following abbreviations were used to assign the proton in NMR spectra: Qn, quinine; Np, naphthalene; Nq, naphthoquinone; Ph, phenyl.

General Procedure 1 (GP1) for the Synthesis of 2−5. A mixture of the ketone (6 mmol, 1.2 equiv), Ti(O^{i}Pr)$_4$ (10 mmol, 2 equiv), and *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (5 mmol, 1 equiv) in 15 mL of anhydrous THF was stirred for 8 h at room temperature under N_2. $NaBH_4$ (15 mmol, 3 equiv) and absolute ethanol were added, and the resulting mixture was stirred for 15 h. The mixture was then poured into 20 mL of 2 M NH_4OH, and the resulting inorganic precipitate was filtered and washed with Et_2O (50 mL).

***N*′-(7-Chloroquinolin-4-yl)-*N*-isopropylethane-1,2-diamine 2.** GP1 from *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (1.3 g, 5.8 mmol), acetone (0.51 mL, 7.0 mmol), Ti(O^{i}Pr)$_4$ (3.50 mL, 11.6 mmol), and $NaBH_4$ (0.66 g, 17.4 mmol) yielded *N*′-(7-chloroquinolin-4-yl)-*N*-isopropylethane-1,2-diamine **2** (0.67 g, 44%) as a colorless solid following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol 4:1). ^{1}H NMR (300 MHz, CD_3OD): δ 8.33 (d, 1H, H-2, ^3J = 5 Hz), 8.05 (d, 1H, H-5, J = 9 Hz), 7.75 (d, 1H, H-8, J = 2 Hz), 7.37 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.52 (d, 1H, H-3, ^3J = 5 Hz), 3.46 (t, 2H, NCH_2, ^3J = 6 Hz) 2.91 (t, 2H, CH_2N^tBu, ^3J = 6 Hz), 2.89 (sept, 1H, CH, ^3J = 6 Hz), 1.09 (d, 6H, CH_3, ^3J = 6 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 152.7, 152.5, 149.7, 136.4, 127.6, 126.1, 124.3, 118.8, 99.7, 49.8, 46.0, 43.6, 22.5. MS (ES+): 263 (M^+). Anal. ($C_{14}H_{18}N_3Cl$) C, H, N.

***N*′-(7-Chloroquinolin-4-yl)-*N*-isobutylethane-1,2-diamine 3.** GP1 from *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (1.0 g, 4.5 mmol), isobutyraldehyde (0.39 g, 0.49 mL, 5.4 mmol), Ti(O^{i}Pr)$_4$ (2.68 mL, 9.0 mmol), and $NaBH_4$ (0.51 g, 13.5 mmol) yielded *N*′-(7-chloroquinolin-4-yl)-*N*-isobutylethane-1,2-diamine **3** (0.585 g, 46.5%) as a pale-yellow solid after flash chromatography (SiO_2, CH_2Cl_2/methanol, 4:1), mp 116 – 118 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.43 (d, 1H, H-2, ^3J = 5 Hz), 7.86 (d, 1H, H-8, J = 2 Hz), 7.64 (d, 1H, H-5, J = 9 Hz), 7.27 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.29 (d, 1H, H-3, ^3J = 5 Hz), 6.01 (s, 1H, NH), 3.25 (t, 2H, $N_{Qn}CH_2$, ^3J = 6 Hz), 2.96 (t, 2H, CH_2N, ^3J = 6 Hz), 2.41 (d, 2H, CH_2, ^3J = 7 Hz), 2.10 (s, 1H, NH), 1.70 (m, 1H, CH), 0.50 (m, 2H, CH_2), 0.87 (d, 6H, ^{i}Pr). MS (FAB+): 278.1 (M^+ + 1). Anal. ($C_{15}H_{20}ClN_3$) C, H, N.

***N*′-(7-Chloroquinolin-4-yl)-*N*-(2,2-dimethylpropyl)ethane-1,2-diamine 4.** GP1 from *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (0.20 g, 0.9 mmol), trimethylacetaldehyde (0.12 mL, 1.1 mmol), Ti(O^{i}Pr)$_4$ (0.54 mL, 1.8 mmol), and $NaBH_4$ (0.10 g, 2.7 mmol) yielded *N*′-(7-chloroquinolin-4-yl)-*N*-(2,2-dimethylpropyl)ethane-1,2-diamine **4** (130 mg, 0.44 mmol, 40%) as a pale-yellow solid after flash chromatography (SiO_2, CH_2Cl_2/methanol, 4:1), mp 139–141 °C. ^{1}H NMR (250 MHz, CD_3OD): δ 8.38 (d, 1H, H-2, ^3J = 5 Hz), 8.08 (d, 1H, H-5, J = 9 Hz), 7.80 (d, 1H, H-8, J = 2 Hz), 7.39 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.60 (d, 1H, H-3, ^3J = 5 Hz), 3.51 (t, 2H, $N_{Qn}CH_2$, ^3J = 6 Hz), 2.97 (t, 2H, CH_2N, ^3J = 6 Hz), 2.45 (s, 2H, CH_2), 0.97 (s, 9H, ^{t}Bu). MS (FAB+): 292.1 (M^+ + 1). Anal. ($C_{16}H_{22}ClN_3 \cdot 0.5H_2O$) C, H, N.

***N*′-(7-Chloroquinolin-4-yl)-*N*-cyclopropylmethylethane-1,2-diamine 5.** GP1 from *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (0.20 g, 0.9 mmol), cyclopropanecarboxaldehyde (0.10 mL, 1.1 mmol), Ti(O^{i}Pr)$_4$ (0.54 mL, 1.8 mmol), and $NaBH_4$ (0.10 g, 2.7 mmol) yielded *N*′-(7-chloroquinolin-4-yl)-*N*-cyclopropylmethylethane-1,2-diamine **5** (60 mg, 24%) as a pale-yellow solid after flash chromatography (SiO_2, CH_2Cl_2/methanol, 4:1), mp 117–118 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.35 (d, 1H, H-2, ^3J = 5 Hz), 8.09 (d, 1H, H-5, J = 9 Hz), 7.76 (d, 1H, H-8, J = 2 Hz), 7.38 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.55 (d, 1H, H-3, ^3J = 5 Hz), 3.48 (t, 2H, $N_{Qn}CH_2$, ^3J = 6 Hz), 2.95 (t, 2H, CH_2N, ^3J = 6 Hz), 2.50 (d, 2H, CH_2, ^3J = 7 Hz), 0.96 (m, 1H, CH), 0.50 (m, 2H, CH_2), 0.16 (m, 2H, CH_2). MS (FAB+): 276.1 (M^+ + 1). Anal. ($C_{15}H_{18}ClN_3$) C, H, N.

4-(1-Aziridinyl)-7-chloroquinoline 7. $CsOH \cdot H_2O$ (1.61 g, 9.6 mmol) in 25 mL of anhydrous DMF was stirred over molecular sieves (4Å) for 10 min. *tert*-Butylamine (0.35 g, 4.8 mmol) was added and the reaction mixture stirred for 30 min. Then 4-(2-bromoethylamino)-7-chloroquinoline **6** (1.5 g, 5.8 mmol) was added, and after 20 h the solvent was concentrated in vacuo. The insoluble salt was removed by filtration and washed with ethyl acetate. The filtrate was concentrated in vacuo, treated with 50 mL of 1 N NaOH, and extracted with ethyl acetate (3×). The combined organic layers were washed with brine and dried over Na_2SO_4. Then

the solvent was evaporated. Flash chromatography (SiO_2, ethyl acetate) afforded 4-(1-aziridinyl)-7-chloroquinoline **7** (1.11 g, 5.4 mmol, 93%) as a colorless solid, mp 99–100 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (d, 1H, H-2, ^3J = 5 Hz), 8.23 (d, 1H, H-5, J = 9 Hz), 8.08 (d, 1H, H-8, J = 2 Hz), 7.52 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.85 (d, 1H, H-3, ^3J = 5 Hz), 2.40 (s, 4H, 2 × CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.1, 151.6, 149.1, 135.1, 128.4, 126.4, 123.8, 122.1, 109.9, 27.9. MS (FAB+): 205.0 (M^+ + 1). Anal. ($C_{11}H_9ClN_2$) C, H, N.

***N*-*tert*-Butyl-2-(quinolin-4-ylamino)acetamide 8.** *N*-α-(*tert*-Butoxycarbonyl)glycin-*tert*-butylamide (1.20 g, 5.2 mmol) and trifluoracetic acid (4.7 mL, 35 mmol) in 4.7 mL of CH_2Cl_2 were stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo, 10 N NaOH (1 mL) was added to the residual oil, and again the mixture was evaporated. The resulting crude 2-amino-*N*-*tert*-butylacetamide was used without further purification for the following reaction. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.83 (t, 2H, CH_2), 2.69 (t, 2H, CH_2), 1.95 (s_{br}, 3H, NH_2, NH), 1.16 (s, 9H, ^{t}Bu). 4,7-Dichloroquinoline (1.02 g, 5.2 mmol) and the crude 2-amino-*N*-*tert*-butylacetamide were heated to reflux overnight. After the mixture was cooled to room temperature, the crude product was filtered and washed with warm water. After flash chromatograpy (SiO_2, CH_2Cl_2/methanol, 12:1) *N*-*tert*-butyl-2-(quinolin-4-ylamino)acetamide **8** (950 mg, 3.3 mmol, 63%) was obtained as a colorless solid, mp 185 °C. ^{1}H NMR (250 MHz, CD_3OD): δ 8.39 (d, 1H, H-2, ^3J = 5 Hz), 8.20 (d, 1H, H-5, J = 9 Hz), 7.80 (d, 1H, H-8, J = 2 Hz), 7.50 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.52 (d, 1H, H-3, ^3J = 5 Hz), 4.09 (s, 2H, CH_2), 1.37 (s, 9H, *t*Bu). MS (ES+): 292 (M^+ + 1), 157.

***N*′-(7-Chloroquinolin-4-yl)-*N*-*tert*-butylethane-1,2-diamine 9.** To *N*-*tert*-butyl-2-(quinolin-4-ylamino)acetamide **8** (0.15 g, 0.51 mmol) in 1 mL of anhydrous THF was added dropwise a 1 M BH_3/THF solution (5.1 mL) under N_2 at 0 °C. After the mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h, the excess of BH_3 was hydrolyzed by 1 mL of 1 M $NaHCO_3$ and the solvent was evaporated. The residue was treated with brine and extracted with CH_2Cl_2. The combined organic layers were evaporated. The residue was treated with 20 mL of THF, and the insoluble salt was removed by filtration and washed with THF. The solution was concentrated to about 20 mL and then 4 N HCl (0.5 mL) was added. After the mixture was stirred for 2 h, the solvent was evaporated and the residue was treated with 1 N NaOH and extracted with CH_2Cl_2. After evaporation of the solvent the *N*′-(7-chloroquinolin-4-yl)-*N*-*tert*-butylethane-1,2-diamine **9** was purified by preparative TLC (SiO_2, CH_2Cl_2/methanol, 6:1) and obtained as a colorless solid (32 mg, 0.11 mmol, 22%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.37 (d, 1H, H-2, ^3J = 5 Hz), 8.10 (d, 1H, H-5, J = 9 Hz), 7.78 (d, 1H, H-8, J = 2 Hz), 7.41 (dd, 1H, H-6, J = 2 Hz, J = 9 Hz), 6.58 (d, 1H, H-3, ^3J = 5 Hz), 3.50 (t, 2H, NCH_2, ^3J = 6 Hz), 2.94 (t, 2H, CH_2N^tBu, ^3J = 6 Hz), 1.17 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, CD_3OD): δ 152.7, 152.5, 149.7, 136.5, 127.7, 126.2, 124.3, 118.8, 99.8, 52.4, 43.8, 41.6, 28.4. MS (ES+): 278 (M^+).

1,4-Dimethoxy-2-methyl-3-trifluoromethylnaphthalene 12. 2-Bromo-3-methyl-1,4-dimethoxynaphthalene **11** (9.13 g, 32.54 mmol), sodium trifluoracetate (13.95 g, 102.56 mmol), and copper iodide (13.02 g, 68.37 mmol) were suspended under argon in 40 mL of dry toluene in a three-necked flask equipped with a Dean–Stark apparatus and a thermometer. About one-third of toluene was distilled off to remove traces of water. After addition of 50 mL of dry dimethylacetamide, the solvent was distilled until the temperature of the mixture increased to 170 °C. After the mixture was stirred for 8 h at this temperature, the solvent was evaporated at 75 °C. CH_2Cl_2 and then petroleum ether were added, and the subsequent precipitated copper iodide was filtered out through silica gel. The precipitate was washed with CH_2Cl_2/petroleum ether (1:2), and then the solvent was removed in vacuo. Flash chromatography (SiO_2, CH_2Cl_2/petroleum ether, 1:5) yielded pure 1,4-dimethoxy-2-methyl-3-trifluoromethylnaphthalene **12** (4.10 g, 15.17 mmol, 46%). A fraction of the starting bromide **11** (3.02 g, 33%) was also isolated. The product **12** was recrystallized from methanol/water, mp 46–47 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.15 (d, 1H, Ar−*H*, J = 8.0 Hz), 8.08 (d, 1H, Ar−*H*, J = 8.0 Hz), 7.57 (m, 2H, Ar−*H*), 3.96 (s, 3H, −OC*H*$_3$), 3.86 (s, 3H, −OC*H*$_3$), 2.52 (q, 3H, ^5J = 2.7 Hz, −CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.7, 150.9, 130.4, 128.4, 127.7, 126.4, 124.7 (q, ^1J = 275.8 Hz, *C*F_3), 124.5, 123.7, 123.6, 122.9, 122.6, 122.3, 119.0, 79.0, 63.9, 61.4, 12.8. MS (FAB+): 270.1 (M^+). Anal. ($C_{14}H_{13}F_3O_2$) C, H.

2-Bromomethyl-1,4-dimethoxy-3-trifluoromethyl-naphthalene 13. To a solution of 1,4-dimethoxy-2-methyl-3-trifluoromethylnaphthalene (1.34 g, 5.0 mmol) in 18 mL of CCl_4 was added *N*-bromosuccinimide (0.89 g, 5.0 mmol) and then benzoyl peroxide (0.12 g, 0.5 mmol). After the mixture was heated to reflux for 5 h and cooled to room temperature, the succinimide was filtered off and the filtrate was washed with sodium dithionite and 2 N NaOH and then dried over $MgSO_4$. After evaporation of the solvent the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/petroleum ether, 1:3) to give 2-bromomethyl-1,4-dimethoxy-3-trifluoromethylnaphthalene **13** (0.97 g, 2.78 mmol, 55%) as colorless crystals after recrystallization from methanol/water, mp 72–73 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.22 (dd, 1H, Ar−*H*, J = 7.3 and 1.8 Hz), 8.15 (dd, 1H, Ar−*H*, J = 7.3 and 1.8 Hz), 7.68 (m, 2H, Ar−*H*), 4.94 (s, 2H, C*H*$_2$Br), 4.14 (s, 3H, −OC*H*$_3$), 4.04 (s, 3H, −OC*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.5, 152.7, 130.2, 129.4, 128.9, 127.9, 124.5, 124.4 (q, ^1J = 275.9 Hz, *C*F_3), 123.9, 123.2, 117.9 (q, ^2J = 29.09 Hz, *C*−CF_3), 79.0, 64.2, 62.9, 24.5. MS (FAB+): calcd 349.0 (M^+); found 348.0, 350.0 (M^+). Anal. ($C_{14}H_{12}BrF_3O_2$) C, H, Br.

7-Chloro-*N*-(3-((diethylamino)methyl)-4-((1,4-dimethoxy-3-(trifluoromethyl)naphthalen-2-yl)methoxy)phenyl)quinolin-4-amine 14. To a solution of amodiaquine dihydrochloride (256 mg, 0.55 mmol) in dry DMF (17 mL) was added, under argon, cesium hydroxide hydrate (360 mg, 2.14 mmol) and then powdered molecular sieves (4 Å). After the mixture was stirred at room temperature for 40 min, the bromide **13** (160 mg, 0.46 mmol) in 5 mL of DMF was added dropwise. After being further stirred for 6 h, the brown reaction mixture was poured into 2 N NaOH solution (100 mL). A yellow solid precipitated. Then the mixture was extracted with CH_2Cl_2 (2×) and the combined organic layers were washed with brine and dried over $MgSO_4$. The solvent and DMF were removed in vacuo and the crude product was purified by flash chromatography on SiO_2 (CH_2Cl_2/methanol, 10:1) and then recrystallized from methanol/water to give 7-chloro-*N*-(3-((diethylamino)methyl)-4-((1,4-dimethoxy-3-(tri-fluoromethyl)naphthalen-2-yl)methoxy)phenyl)quinolin-4-amine **14** (234 mg, 0.37 mmol, 82%) as pale-yellow crystals, mp 161–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49 (d, 1H, QnH-2, ^3J = 5 Hz), 8.21 (m, 1H, NpH-5/8), 8.20 (m, 1H, NpH-5/8), 8.00 (d, 1H, QnH-8, J = 2 Hz), 7.85 (d, 1H, QnH-5, ^3J = 9 Hz), 7.69 (m, 2H, NpH-6/7), 7.46 (d, 1H, J = 3 Hz, PhH-2′), 7.43 (dd, 1H, ^2J = 9 Hz, ^3J = 2 Hz, QnH-6), 7.19 (dd, 1H, J = 3 Hz, J = 9 Hz, PhH-6′), 7.15 (d, 1H, J = 9 Hz, PhH-5′), 6.82 (d, 1H, QnH-3, ^3J = 5 Hz), 6.62 (s, 1H, NH), 5.38 (d, 1H, OCH_2, J = 0.9 Hz), 4.05 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 3.53 (s, 2H, NCH_2), 2,46 (q, 4H, 2 × CH_2, ^3J = 7 Hz), 0.93 (t, 6H, 2 × CH_3, ^3J = 7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.6, 153.5, 153.2, 152.0, 149.7, 148.8, 135.1, 131.9, 130.8, 130.4, 129.5, 129.0, 128.8, 127.9, 126.0, 124.4 (q, ^1J = 275.3 Hz, *C*F_3), 125.7, 123.8, 123.2, 123.1, 122.5, 121.0, 119.2 (q, ^2J = 30 Hz, *C*-CF_3), 111.8, 101.6, 64.1, 62.1, 62.0, 50.6, 47.4, 12.0. MS (FAB+): 623 (M^+), 552, 354, 269, 200. Anal. ($C_{34}H_{33}ClF_3N_3O_3 \cdot 1H_2O$) C, H, N.

***N*-[4-(1,4-Dimethoxy-3-trifluoromethylnaphthalen-2-yl-methoxy) phenyl]acetamide 15.** To 4-acetamidophenol (178 mg, 1.14 mmol) in dry DMF (7.5 mL) was added, under argon, cesium hydroxide hydrate (400 mg, 2.38 mmol) and then powdered molecular sieves (4 Å). After the mixture was stirred at 40 °C for 30 min and cooled to room temperature, the bromide **13** (200 mg, 0.57 mmol) in 2.5 mL of DMF was added dropwise. The reaction mixture was stirred at 40 °C for a further 6 h and 30 min. Then CH_2Cl_2 was added, and the mixture was poured into brine. Phase separation, extraction of the aqueous layer with CH_2Cl_2 (3×), drying of the combined organic layers over $MgSO_4$, and evaporation of

the solvent and DMF in vacuo led to the product, which was purified by flash chromatography (SiO_2, CH_2Cl_2/methanol, 100:1) and then recrystallized from methanol/water to afford *N*-[4-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethoxy)phenyl]acetamide **15** (223 mg, 0.53 mmol, 93%) as colorless crystals, mp 156–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (m, 2H, NpH-5/8), 7.63 (m, 2H, NpH-6/7), 7.40 (m, 2H, PhH-2/6), 6.97 (m, 2H, PhH-3/5), 5.29 (s, 2H, OCH_2), 4.00 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.7, 156.1, 153.7, 131.8, 130.8, 129.9, 129.1, 128.2, 126.6, 124.2, 123.5, 123.4, 123.0, 122.4, 119.6, 115.5, 105.7, 64.4, 62.63, 62.6, 24.7. MS (EI): 419 (M^+), 269, 200, 185, 151. Anal. ($C_{22}H_{20}F_3NO_4$) C, H, N.

General Procedure 2 (GP2) for the Reductive Amination of 1,4-Dimethoxynaphthalene-2-carbaldehyde and 3-Methyl-1,4-dimethoxynaphthalene-2-carbaldehyde with (7-Chloroquinolin-4-yl)ethane-1,2-diamine. (7-Chloroquinolin-4-yl)ethane-1,2-diamine **1** (1 equiv) and aldehyde (1.00 g, 1 equiv) were dissolved in 50 mL of dry CH_2Cl_2 and 5 mL of dry methanol and stirred under argon atmosphere at room temperature. After 2 h, sodium triacetoxyborohydride (3 equiv) was added, and the reaction mixture was stirred for another 24 h. Then the mixture was poured into 100 mL of 2 N NaOH solution, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated in vacuo. Flash chromatography (SiO_2, CH_2Cl_2/methanol, 5:1) afforded the products as pale-yellow oils.

***N*-(7-Chloroquinolin-4-yl)-*N*′-(1,4-dimethoxynaphthalen-2-ylmethyl)ethane-1,2-diamine 19.** GP2 from 1,4-dimethoxynaphthalene-2-carboxaldehyde **17** and *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine **1** afforded *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxynaphthalen-2-ylmethyl)ethane-1,2-diamine **19** (0.86 g, 2.04 mmol, 44%) as a pale-yellow foam, mp 48–50 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, 1H, QnH-2, ^3J = 5 Hz), 8.27 (m, 1H, NpH-5/8, J = 8 Hz), 8.06 (m, 1H, NpH-5/8, J = 8 Hz), 7.96 (d, 1H, QnH-8, J = 2 Hz), 7.69 (d, 1H, QnH-5, J = 9 Hz), 7.55 (m, 2H, NpH-6/7), 7.34 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.78 (s, 1H, NpH-3), 6.40 (d, 1H, QnH-3, ^3J = 5 Hz), 5.91 (s_{br}, 1H, NH), 4.06 (s, 2H, NCH_2Np), 3.98 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.38 (m, 2H, $N_{Qn}CH_2$), 3.10 (m, 2H, CH_2N), 2.00 (s_{br}, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.0, 151.6, 149.7, 148.6, 147.4, 134.7, 128.3, 127.0, 126.6, 126.0, 125.3, 125.0, 122.2, 121.5, 121.1, 117.1, 104.5, 99.9, 62.2, 55.5, 47.7, 46.4, 41.7. MS (FAB+): 422 (M^+). Anal. ($C_{24}H_{24}ClN_3O_2 \cdot 0.5H_2O$) C, H, N.

***N*-(7-Chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)ethane-1,2-diamine 20.** GP2 from 1,4-dimethoxy naphthalene-3-methyl-2-naphthalenecarboxaldehyde **18** and *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine **1** afforded *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)ethane-1,2-diamine **20** (1.71 g, 3.92 mmol, 90%) as a pale-yellow foam, mp 51–53 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, 1H, QnH-2, ^3J = 5 Hz), 8.08 (m, 2H, NpH-5/8), 7.96 (d, 1H, QnH-8, J = 2 Hz), 7.67 d, 1H, QnH-5, J = 9 Hz), 7.53 (m, 2H, NpH-6/7), 7.34 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.39 (d, 1H, QnH-3, ^3J = 5 Hz), 5.92 (s_{br}, 1H, NH), 4.06 (s, 2H, NCH_2Np), 3.94 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.38 (m, 2H, $N_{Qn}CH_2$), 3.13 (m, 2H, CH_2N), 2.53 (CH_3), 1.77 (s_{br}, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.0, 150.9, 150.5, 149.9, 149.1, 134.8, 128.6, 128.4, 128.3, 127.1, 126.3, 126.2, 125.6, 125.2, 122.4, 122.3, 121.2, 117.3, 99.2, 62.8, 61.3, 47.1, 44.7, 41.8), 12.3 (CH_3). MS (FAB+): 436 (M^+), 215. Anal. ($C_{25}H_{26}ClN_3O_2 \cdot 0.5H_2O$) C, H, N.

***N*-(7-Chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine 21.** To (7-chloroquinolin-4-yl)ethane-1,2-diamine **1** (2.90 g, 13.06 mmol) in dry DMF (70 mL) and anhydrous ethanol (20 mL) was added triethylamine (1.32 g, 1.82 mL, 13.06 mmol) and then 2-bromomethyl-1,4-dimethoxy-3-trifluoromethylnaphthalene **13** (1.52 g, 4.35 mmol) in dry DMF (5 mL) under argon atmosphere. After being stirred for 18 h at room temperature, the reaction mixture was poured into brine, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (SiO_2, CH_2Cl_2/methanol, 5:1) yielded *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21** (1.81 g, 3.70 mmol, 85%) as a pale-yellow oil/foam. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.51 (d, 1H, QnH-2, ^3J = 5 Hz), 8.21 (m, 1H, NpH-5/8), 8.08 (m, 1H, NpH-5/8), 7.95 (d, 1H, QnH-8, J = 2 Hz), 7.73 (d, 1H, QnH-5, J = 9 Hz), 7.68 (m, 2H, NpH-6/7), 7.34 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.38 (d, 1H, QnH-3, ^3J = 5 Hz), 6.05 (s_{br}, 1H, NH), 4.15 (s, 2H, NCH_2Np), 4.01 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.35 (m, 2H, $HNCH_2$), 3.11 (m, 2H, CH_2N), 2.10 (s_{br}, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.2, 152.0, 151.7, 150.0, 148.7, 134.7, 130.1, 128.7, 128.6, 128.2, 127.2, 126.5, 126.1, 125.0, 124.7 (q, $-CF_3$), 123.7, 122.6, 121.5, 118.2 (q, $C-CF_3$), 117.3, 99.0, 64.0, 62.8, 46.9, 44.2, 41.7. MS (EI): 488.9 (M^+), 269, 192, 156.

Preparation of the Dihydrochloride Salt 21. To a solution of *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21** (1.40 g, 2.86 mmol) in methanol (10 mL) was added trimethylchlorosilane (5.72 mmol, 0.73 mL, 2 equiv). The reaction mixture was stirred for 15 min at room temperature. Then the solid was filtered and washed with methanol and diethyl ether to give the dihydrochloride salt of *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21** (1.05 g, 1.86 mmol, 65%), mp 213–215 °C. A second batch was obtained by further addition of diethyl ether to the filtrate. Anal. ($C_{25}H_{23}ClF_3NO_2 \cdot 2HCl \cdot 1H_2O$) C, H, N.

Geneal Procedure 3 (GP3) for the Reductive Amination of the Chloroquinoyl-1,4-dimethoxynaphthalene Derivatives 22–30 with Acetone, Isobutyraldehyde, or Pivalaldehyde. The amine derivative (1 equiv) and the ketone or aldehyde (14–30 equiv of acetone, 3–6 equiv of isobutyraldehyde, or 6 equiv of pivalaldehyde) in dry CH_2Cl_2 (10–30 mL) were stirred under argon atmosphere at room temperature for 2 h and 30 min. Then sodium triacetoxyborohydride (3 equiv) was added and the reaction mixture was stirred for further 2–3 days. If isobutyraldehyde is used, the reaction time is 2–5 h. The reaction mixture was poured into 2 N NaOH solution, the organic layer separated, and the aqueous layer extracted with CH_2Cl_2 (3×). After drying of the combined organic layers over $MgSO_4$, the solvent was removed in vacuo and the crude product purified by flash chromatography (SiO_2, CH_2Cl_2/methanol, 1:1).

***N*-(7-Chloroquinolin-4-yl)-*N*′-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine 22.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxynaphthalen-2-ylmethyl)ethane-1,2-diamine **19** (0.25 g, 0.59 mmol), acetone (0.48 g, 0.61 mL, 8.3 mmol), and $NaBH(OAc)_3$ (0.38 g, 1.78 mmol) after 48 h yielded *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine **22** (0.27 g, 0.58 mmol, 98%) as a colorless oil, mp 139–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (d, 1H, QnH-2, ^3J = 5 Hz), 8.22 (m, 1H, NpH-5/8, J = 8 Hz), 8.02 (m, 1H, NpH-5/8, J = 8 Hz), 7.93 (d, 1H, QnH-8, J = 2 Hz), 7.59–7.47 (m, 2H, NpH-6/7), 7.44 (d, 1H, QnH-5, J = 9 Hz), 7.27 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.78 (s, 1H, NpH-3), 6.27 (d, 1H, QnH-3, ^3J = 5 Hz), 5.88 (s_{br}, 1H, NH), 3.88 (s, 3H, OCH_3), 3.83 (s, 2H, NCH_2Np), 3.80 (s, 3H, OCH_3), 3.22 (m, 2H, $N_{Qn}CH_2$), 3.11 (sept, 1H, CH, ^3J = 7 Hz), 2.96 (m, 2H, CH_2N), 1.20 (d, 6H, CH_3, ^3J = 7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.2, 151.6, 150.0, 148.6, 148.0, 134.9, 128.6, 128.2, 127.3, 126.8, 126.1, 125.5, 125.1, 122.4, 121.7, 121.1, 117.2, 105.0, 99.0, 62.4, 55.4, 49.8, 47.7, 47.0, 40.2, 18.1. MS (FAB+): 464 (M^+). Anal. ($C_{27}H_{30}ClN_3O_2 \cdot 0.5H_2O$) C, H, N.

***N*′-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine 23.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)ethane-1,2-diamine **20** (0.40 g, 0.92 mmol), acetone (1.60 g, 2.0 mL, 27.5 mmol), and $NaBH(OAc)_3$ (0.59 g, 2.80 mmol) after 66 h yielded *N*′-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine **23** (0.35 g, 0.73 mmol, 80%) as a colorless solid, mp 147–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, 1H, QnH-2, ^3J = 5 Hz), 8.10–8.00 (m, 2H, NpH-5/8), 7.92 (d, 1H, QnH-8, J = 2 Hz), 7.55–7.47 (m, 2H, NpH-6/7), 7.46 (d, 1H, QnH-5, J = 9 Hz), 7.36

(dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.11 (d, 1H, QnH-3, $^3J = 5$ Hz), 5.64 (s_{br}, 1H, NH), 3.90 (s, 3H, OCH_3), 3.88 (s, 2H, NCH_2Np), 3.78 (s, 3H, OCH_3), 3.10 (sept, 1H, CH, $^3J = 7$ Hz), 3.07 (m, 2H, $N_{Qn}CH_2$), 2.88 (m, 2H, CH_2N), 2.54 (s, 3H, CH_3), 1.20 (d, 6H, CH_3, $^3J = 7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.6, 151.5, 150.5, 150.0, 148.6, 134.8, 128.4, 128.1, 127.5, 127.1, 127.0, 126.2, 125.6, 125.3, 122.4, 122.3, 121.3, 117.2, 98.9, 62.6, 61.2, 50.7, 46.3, 45.7, 40.6, 18.0, 12.4. MS (FAB+): 478 (M^+). Anal. ($C_{28}H_{32}ClN_3O_2 \cdot 0.5H_2O$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine 24.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21** (0.40 g, 0.81 mmol)), acetone (1.80 mL, 1.42 g, 24.5 mmol), and $NaBH(OAc)_3$ (0.52 g, 2.50 mmol) after a 6-day-long reaction yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isopropylethane-1,2-diamine **24** (0.38 g, 7.14 mmol, 87%) following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol, 20:1) and recrystallization from diethyl ether/petroleum ether (colorless crystals), mp 87–88 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.34 (d, 1H, QnH-2, $^3J = 5$ Hz), 8.05 (m, 2H, NpH-5/8), 7.92 (d, 1H, QnH-8, $J = 2$ Hz), 7.71 (d, 1H, QnH-5, $J = 9$ Hz), 7.60 (m, 2H, NpH-6/7), 7.38 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.09 (d, 1H, QnH-3, $^3J = 5$ Hz), 5.93 (s_{br}, 1H, NH), 4.09 (s, 2H, CH_2Np), 3.93 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.00–2.94 (m, 3H, $HNCH_2 + CH$), 2.81 (m, 2H, CH_2N), 1.10 (d, 6H, $^3J = 6$ Hz, $2 \times CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.9, 152.5, 151.7, 150.0, 148.9, 134.7, 130.1, 128.7, 128.6, 128.2, 127.3, 126.6, 125.8, 125.2 (q, $^1J = 270$ Hz, CF_3), 125.0, 123.7, 122.9, 122.7, 121.6, 119.0 (q, $^2J = 30$ Hz, $C-CF_3$), 117.5, 98.9, 63.9, 62.8, 50.8, 46.7, 46.4, 40.2, 17.9. MS (FAB+): 532 (M^+), 340. Anal. ($C_{28}H_{29}F_3ClN_3O_2$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine 25.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxynaphthalen-2-ylmethyl)ethane-1,2-diamine **19** (0.25 g, 0.59 mmol), isobutyraldehyde (0.24 g 3.30 mmol), and $NaBH(OAc)_3$ (0.38 g, 1.78 mmol) after 2 h yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine **25** (0.28 g, 0.59 mmol, 99%) as a pale-yellow oil, mp 48–49 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (d, 1H, QnH-2, $^3J = 5$ Hz), 8.26 (m, 1H, NpH-5/8, $J = 8$ Hz), 8.05 (m, 1H, NpH-5/8, $J = 8$ Hz), 7.94 (d, 1H, QnH-8, $J = 2$ Hz), 7.61–7.46 (m, 2H, NpH-6/7), 7.53 (d, 1H, QnH-5, $J = 9$ Hz), 7.32 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.83 (s, 1H, NpH-3), 6.29 (d, 1H, QnH-3, $^3J = 5$ Hz), 5.78 (s_{br}, 1H, NH), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.82 (s, 2H, NCH_2Np), 3.27 (m, 2H, $N_{Qn}CH_2$), 2.88 (m, 2H, CH_2N), 2.38 (d, 2H, CH_2, $^3J = 7$ Hz), 2.00 (m, 1H, CH, $^3J = 7$ Hz), 0.95 (d, 6H, $2 \times CH_3$, $^3J = 7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.1, 151.8, 149.9, 148.8, 148.1, 134.8, 128.6, 128.5, 126.9, 126.8, 126.2, 125.6, 125.1, 122.4, 121.8, 121.2, 117.3, 105.3, 99.0, 63.3, 62.4, 55.6, 53.3, 52.4, 40.2, 26.5, 18.8. MS (FAB+): 478 (M^+). Anal. ($C_{28}H_{32}Cl_3N_3O_2$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine 26.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)ethane-1,2-diamine **20** (0.40 g, 0.92 mmol), isobutyraldehyde (0.20 g, 0.25 mL, 2.75 mmol), and $NaBH(OAc)_3$ (0.59 g, 2.80 mmol) after a 5-h-long reaction yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine **26** (0.44 g, 0.89 mmol, 97%) as colorless crystals following recrystallization (diethylether/petroleum ether), mp 88–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (d, 1H, QnH-2, $^3J = 5$ Hz), 8.14–8.04 (m, 2H, NpH-5/8), 7.92 (d, 1H, QnH-8, $J = 2$ Hz), 7.55 (d, 1H, QnH-5, $J = 9$ Hz), 7.56–7.48 (m, 2H, NpH-6/7), 7.40 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.20 (d, 1H, QnH-3, $^3J = 5$ Hz), 5.62 (s_{br}, 1H, NH), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.86 (s, 2H, NCH_2Np), 3.16 (m, 2H, $N_{Qn}CH_2$), 2.81 (m, 2H, CH_2N), 2.59 (s, 3H, CH_3), 2.37 (d, 2H, CH_2, $^3J = 6$ Hz), 1.92 (m, 1H, CH), 0.88 (d, 6H, CH_3, $^3J = 6$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.5, 151.4, 150.4, 149.7, 148.5, 134.6, 128.3, 128.1, 126.9, 126.8, 126.7, 126.1, 125.5, 125.0, 122.3, 122.1, 121.2, 117.0, 98.7, 63.9, 62.3, 61.1, 51.7, 51.0, 40.2, 26.2, 20.8, 12.4. MS (FAB+): 492 (M^+). Anal. ($C_{29}H_{34}ClN_3O_2 \cdot 0.5Et_2O$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine 27.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21** (0.40 g, 0.81 mmol), isobutyraldehyde (0.22 mL, 0.18 g, 2.5 mmol), and $NaBH(OAc)_3$ (0.52 g, 2.50 mmol) after a 6-day-long reaction yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isobutylethane-1,2-diamine **27** (0.40 g, 7.32 mmol, 90%) as colorless crystals following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol, 20:1) and recrystallization from diethylether/petroleum ether, mp 91–92 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (d, 1H, QnH-2, $^3J = 5$ Hz), 8.14 (d, 1H, $J = 8$ Hz, NpH-5/8), 8.06 (d, 1H, $J = 8$ Hz, NpH-5/8), 7.91 (d, 1H, QnH-8, $J = 2$ Hz), 7.80 (d, 1H, QnH-5, $J = 9$ Hz), 7.66–7.56 (m, 2H, NpH-6/7), 7.38 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.18 (d, 1H, QnH-3, $^3J = 5$ Hz), 6.00 (s_{br}, 1H, NH), 3.98 (s, 2H, NCH_2Np), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.15 (m, 2H, $HN_{Qn}CH_2$), 2.73 (m, 2H, CH_2N), 2.24 (d, 2H, CH_2, $J = 7$ Hz), 1.73 (m, 1H, CH), 0.76 (d, 6H, $^3J = 6$ Hz, $2 \times CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.8, 152.6, 151.6, 150.1, 148.8, 134.8, 130.2, 128.8, 128.7, 128.2, 127.4, 127.3 (q, $^1J = 270$ Hz, CF_3), 126.5, 125.2, 125.0, 123.8, 122.7, 121.7, 119.3 (q, $^2J = 28.3$ Hz, C-CF_3), 117.4, 98.9, 64.1, 63.6, 62.5, 52.5, 51.1, 40.2, 26.3, 20.9. MS (FAB+): 546 (M^+), 532, 354. Anal. ($C_{29}H_{31}ClF_3N_3O_2$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine 28.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxynaphthalen-2-ylmethyl)ethane-1,2-diamine **19** (0.16 g 0.37 mmol), pivalaldehyde (0.25 mL, 2.28 mmol), and $NaBH(OAc)_3$ (0.25 g, 1.14 mmol) after an 80-min-long reaction yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxynaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine **28** (0.18 g, 0.37 mmol, 97%) as a pale-yellow oil following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol, 40:1), mp 53–56 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.37 (d, 1H, QnH-2, $^3J = 5$ Hz), 8.23 (m, 1H, NpH-5/8), 7.99 (m, 1H, NpH-5/8), 7.92 (d, 1H, QnH-8, $J = 2$ Hz), 7.50 (m, 3H, NpH-6/7, QnH-5), 7.33 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.91 (s, 1H, NpH-3), 6.22 (d, 1H, QnH-3, $J = 5$ Hz), 5.75 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 3.84 (s, 2H, $NpCH_2$), 3.81 (s, 3H, OCH3), 3.18 (m, 2H, NCH_2), 2.85 (m, 2H, NCH_2), 2.45 (s, 2H,CH_2tBu), 0.90 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.1, 151.3, 150.1, 148.2, 148.1, 135.0, 128.6, 128.1, 127.1, 126.9, 126.2, 125.6 (NpC-6/7), 125.2, 122.4, 121.8, 121.3, 117.2, 105.4, 98.9, 77.2, 67.8, 62.4, 55.7, 55.2, 54.4, 40.9, 32.9, 28.5. MS (FAB+): 492 (M^+). Salt **28**, mp 178–180 °C. Anal. ($C_{29}H_{34}ClN_3O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine 29.** GP3 from *N*-(7-chloroquinolin-4-yl)-*N′*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)ethane-1,2-diamine **20** (0.21 g, 0.48 mmol), pivalaldehyde (0.25 g, 0.31 mL, 2.89 mmol), and $NaBH(OAc)_3$ (0.31 g, 1.44 mmol) after a 2.5-h-long reaction yielded *N′*-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine **29** (0.21 g, 0.41 mmol, 86%) as a colorless solid following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol, 20:1), mp 63–65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (d, 1H, QnH-2, $^3J = 5$ Hz), 7.98 (m, 2H, NpH-5/8), 7.89 (d, 1H, QnH-8, $J = 2$ Hz), 7.71 (d, 1H, QnH-5, $J = 9.0$ Hz), 7.42 (m, 2H, NpH-6/7), 7.34 (dd, 1H, QnH-6, $J = 2$ Hz, $J = 9$ Hz), 6.16 (d, 1H, QnH-2, $J = 5$ Hz), 6.01 (s, 1H, NH), 3.80 (s, 2H, NCH_2Np), 3.75 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.18 (m, 2H, NCH_2), 2.80 (m, 2H, NCH_2), 2.47 (s, 3H, CH_3), 2.32 (s, 2H, CH_2tBu), 0.72 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.9, 151.3, 150.5, 150.2, 148.4, 135.1, 128.5, 128.0, 127.4, 127.0 126.3, 125.7, 125.4, 122.5, 122.4, 121.7, 117.3, 98.9, 73.5, 67.6, 62.3, 61.3, 54.7, 52.0, 40.7, 32.8, 28.6, 13.2. MS (FAB+): 506 (M^+). Anal. ($C_{30}H_{36}ClN_3O_2 \cdot 0.4H_2O$) C, H, N.

***N′*-(7-Chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine 30.** *N*-(7-Chloroquinolin-4-yl)-*N′*-(1,4-dimethoxy-3-trifluoromethylnaphtha-

len-2-ylmethyl)ethane-1,2-diamine dihydrochloride salt **21** (0.14 g, 0.26 mmol) was treated with 2 N NaOH solution to liberate the free base, and the mixture was subsequently extracted with CH_2Cl_2 (3×). The combined organic phases were dried over $MgSO_4$, and then the solvent was removed in vacuo. The product **21** was used without further purification for the reaction following GP3. The reaction involving the free base *N*-(7-chloroquinolin-4-yl)-*N*′-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)ethane-1,2-diamine **21**, pivalaldehyde (0.17 mL, 0.14 g, 1.57 mmol), and $NaBH(OAc)_3$ (0.17 g, 0.78 mmol) after 3 days yielded *N*′-(7-chloroquinolin-4-yl)-*N*-(1,4-dimethoxy-3-trifluoromethylnaphthalen-2-ylmethyl)-*N*-isopentylethane-1,2-diamine **30** (0.10 g, 0.18 mmol, 68%) as a colorless solid following purification by flash chromatography (SiO_2, CH_2Cl_2/methanol, 40:1), mp 71–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (d, 1H, QnH-2, ^3J = 5 Hz), 8.10 (m, 2H, NpH-5/8), 8.00 (d, 1H, QnH-8, J = 2 Hz), 7.90 (d, 1H, QnH-5, J = 9 Hz), 7.55 (m, 2H, NpH-6/7), 7.40 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.58 (s_{br}, 1H, NH), 6.22 (d, 1H, QnH-3, ^3J = 5 Hz), 3.94 (s, 2H, CH_2Np), 3.90 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.18 (m, 2H, NCH_2), 2.79 (m, 2H, CH_2N), 2.26 (s, 2H, CH_2^{t}Bu), 0.84 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, CDCl3): δ 153.6, 153.5, 152.9, 152.5, 151.1, 135.9, 130.1, 129.0, 128.8, 127.5, 127.0, 125.6, 125.2, 123.9, 122.5, 122.0, 119.2 (q, *C*−CF_3), 117.2, 98.8, 64.1, 62.3, 54.8, 51.4, 40.5, 32.6, 28.5. MS (FAB+): 560 (M^+). Anal. ($C_{30}H_{33}ClF_3N_3O_2\cdot 0.4H_2O$) C, H, N.

***N*-[4-Hydroxy-3-({isopropyl-[3-(7-chloroquinolin-4-ylamino) propyl]amino}methyl)phenyl]acetamide 32.** Acetamidophenol (0.48 g, 3.17 mmol) was subjected to the Mannich reaction with *N*-(7-chloroquinolin-4-yl)propane-3-isopropyl-1,3-diamine **31** (1.77 g, 6.37 mmol) and 37% aqueous formaldehyde (0.65 mL, 8 mmol) in 5 mL of ethanol. After the mixture was heated to reflux for 79 h, the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (10 mL). The organic solution was extracted with dilute hydrochloric acid (0.1 M, 2 × 20 mL). This solution was basified (pH 9–10) with 0.65 mL of 0.1 N NaOH and extracted with dichloromethane (5 × 20 mL). The combined extracts were washed with water (1 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the product as a crude oil, mp 108–109 °C. Flash chromatography (SiO_2, CH_2Cl_2/methanol, 6:1) afforded the product **32** (0.25 g, 0.59 mmol, 18.5%) as colorless crystals. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.44 (d, 1H, QnH-2, ^3J = 5 Hz), 7.91 (d, 1H, QnH-5, J = 2 Hz), 7.63 (d, 1H, QnH-3, J = 9 Hz), 7.54 (s_{br}, 1H, NHCO), 7.37 (d, 1H, PhH-2′, J = 2 Hz), 7.30 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 7.03 (dd, 1H, PhH-6′, J = 2 Hz, J = 9 Hz), 6.74 (d, 1H, PhH-5′, J = 9 Hz), 6.33 (d, 1H, QnH-3, ^3J = 5 Hz), 5.70 (s_{br}, 1H, NH), 3.73 (s, 2H, $PhCH_2N$), 3.35 (t, 2H, NCH_2, ^3J = 6 Hz), 3.15 (sept, 1H, CH, ^3J = 7 Hz), 2.61 (t, 2H, NCH_2, ^3J = 6 Hz), 2.15 (s, 3H, $COCH_3$), 1.93 (t, 2H, CH_2, ^3J = 6 Hz), 1.11 (d, 6H, 2 × CH_3, ^3J = 7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 154.6, 151.1, 149.9, 148.2, 135.1, 129.8, 127.8, 125.3, 122.1, 121.6, 120.9, 117.0, 116.0, 98.7, 52.9, 49.4, 46.6, 40.7, 26.2, 24.3, 17.1. MS (FAB+): 441.1 (M^+ + 1). HRMS (FAB+, NBA): calcd (M^+ + 1) 441.2057; found 441.2043. Anal. ($C_{24}H_{29}ClN_4O_2\cdot 2H_2O$) C, H, N.

General Procedure 4 (GP4) for the Synthesis of Tertiary Amides 34–36, Illustrated with 6-(3-Methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic Acid [2-(7-Chloroquinolin-4-ylamino)ethyl]-*N*-isopropylamide 34. To a solution of 6-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic acid **M$_5$** (3 g, 10.5 mmol, 1 equiv) in DMF (35 mL) was added HOBT (1.4 g, 10.5 mmol, 1 equiv) and EDC (2.1 g, 2.4 mL, 13.65 mmol, 1.3 equiv) dropwise at 0 °C. The reaction mixture was allowed to stir at 0 °C for 1 h. Then the amine *N*′-(7-chloroquinolin-4-yl)-*N*-isopropylethane-1,2-diamine **2** (4.1 g, 15.75 mmol, 1.5 equiv) in 30 mL of DMF was added at 0 °C and the reaction mixture was stirred overnight at room temperature. The reaction mixture was extracted with CH_2Cl_2 and successively washed with brine, 0.1 N HCl, brine, 3% $NaHCO_3$ solution, and brine. The combined organic layers were dried over $MgSO_4$ and evaporated. Purification of the crude product was done by two flash chromatography steps (SiO_2, CH_2Cl_2/CH_3OH, 12:1) yielding 6-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic acid [2-(7-chloroquinolin-4-ylamino)ethyl] amide **34** (3.47 g, 62.1%) as an amorphous yellow powder, mp 65–67 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.27 (d, 1H, QnH-2, ^3J = 6 Hz), 7.94–7.88 (m, 3H, QnH-5, NqH-5/8), 7.69 (d, 1H, QnH-8, J = 2 Hz), 7.66–7.63 (m, 2H, NqH-6/7), 7.38 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.65 (d, 1H, QnH-3, ^3J = 6 Hz), 4.24 (sept, 1H, CH), 3.58–3.52 (m, 2H, $N_{Qn}CH_2$), 3.48–3.43 (m, 2H, CH_2N), 2.52–2.45 (m, 4H, CH_2), 2.05 (s, 3H, CH_3), 1.72–1.67 (m, 2H, CH_2), 1.45–1.42 (m, 4H, CH_2), 1.24 (d, 6H, *i*Pr). ^{13}C NMR (75 MHz, CD_3OD): δ 186.1, 185.6, 176.8, 153.5, 150.8, 148.1, 147.5, 144.4, 137.2, 134.5, 133.3, 127.0, 126.6, 126.2, 124.4, 118.1, 99.6, 50.3, 44.4, 40.3, 34.2, 30.5, 29.4, 27.6, 26.4, 21.4, 12.7. MS (FAB+): 532.2 (M^+). HRMS (FAB+, NBA): calcd (M^+ + 1) 532.2367; found: 532.2396. Anal. ($C_{31}H_{34}ClN_3O_3\cdot 1.2\ H_2O$) C, H, N.

6-(3-Methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic Acid [2-(7-Chloroquinolin-4-ylamino)ethyl]amide 35. GP4 from 6-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic acid **M$_5$** (200 mg, 0.7 mmol) and *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (233 mg, 1.05 mmol) afforded 6-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexanoic acid [2-(7-chloroquinolin-4-ylamino)ethyl]amide **35** (70 mg, 0.05 mmol, 19%) as a yellow solid, mp 58–60 °C. ^{1}H NMR (250 MHz, CD_3OD): δ 8.31 (d, 1H, QnH-2, ^3J = 6 Hz), 8.03 (d, 1H, QnH-5, J = 9 Hz), 7.99–7.94 (m, 2H, NqH-5/8), 7.72–7.68 (m, 3H, QnH-8, NqH-6/7), 7.44 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 6.60 (d, 1H, QnH-3, ^3J = 6 Hz), 3.52 (m, 4H, NCH_2CH_2N), 2.48 (m, 2H, CH_2), 2.23 (m, 2H, CH_2), 2.05 (s, 3H, CH_3), 1.65 (m, 2H, CH_2), 1.39 (m, 4H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.1, 184.6, 176.2, 154.0, 147.0, 145.0, 143.2, 141.6, 138.1, 133.3, 132.0, 128.0, 126.1, 124.8, 122.1, 118.2, 115.8, 111.2, 97.6, 45.7, 38.1, 36.1, 29.4, 28.2, 26.8, 25.3, 12.6. MS (FAB+): 490 (M^+ + 1). HRMS (FAB+, NBA): calcd (M^+ + 1) 490.1897; found 490.1876.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-*N*′-isopropyl-2-[4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylmethyl)phenyl]acetamide 36.** GP4 from [4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylmethyl)phenyl]acetic acid **33** (3 g, 9.36 mmol, 1 equiv) and *N*′-(7-chloroquinolin-4-yl)-*N*-isopropylethane-1,2-diamine **2** (3.70 g, 14.04 mmol, 1.5 equiv) yielded *N*-[2-(7-chloroquinolin-4-ylamino)ethyl]-*N*′-isopropyl-2-[4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylmethyl)phenyl]acetamide **36** (1.23 g, 23.2%) as a yellow solid, mp 157–158 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.28 (d, 1H, QnH-2, ^3J = 6 Hz), 7.96 (m, 2H, NqH-5/8), 7.86 (d, 1H, QnH-5, J = 9 Hz), 7.71–7.65 (m, 3H, QnH-8, NqH-6/7), 7.29 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 7.14 (m, 4H, PhH), 6.62 (d, 1H, QnH-3, ^3J = 6 Hz), 4.21 (sept, 1H, CH), 4.20 (s, 2H, $COCH_2$), 3.94 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.52 (m, 4H, NCH_2CH_2N), 2.80 (s, 3H, CH_3), 1.06 (d, 6H, ^{i}Pr). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.2, 184.5, 173.8, 151.0, 149.9, 147.1, 145.0, 144.4, 136.8, 135.5, 133.5, 132.7, 132.0, 131.9, 129.0, 128.8, 126.7, 126.4, 126.2, 125.7, 122.7, 116.9, 97.8, 49.5, 45.8, 40.9, 39.2, 31.9, 21.1, 13.2 (CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 186.3, 185.8, 174.8, 152.5, 149.6, 146.5, 145.6, 138.4, 136.3, 134.7, 134.6, 134.5, 133.4, 133.3, 130.1, 130.1, 130.0, 127.6, 127.2, 127.1, 126.1, 124.1, 118.5, 99.6, 50.8, 43.8, 41.5, 40.5 (CH_2), 38.9, 32.6, 21.1, 13.2. MS (FAB+): 566.4 (M^+ + 1). HRMS (FAB+, NBA) calcd (M^+ + 1) 566.2210; found 566.2207. Anal. ($C_{34}H_{32}ClN_3O_3\cdot 0.5H_2O$) C, H, N.

General Procedure 5 (GP5) for the Synthesis of Tertiary Amides 39–41 Illustrated with *N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-[4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]-*N*-isopropylacetamide 40. [4-(3-Methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylmethyl)phenyl]acetic acid **33** (3.50 g, 9.99 mmol) in 350 mL of ethanol was heated at 65 °C until most of the compound was dissolved. At 50 °C $SnCl_2$ (7.00 g, 36.92 mmol) in 7 mL of concentrated HCl was added slowly and further stirred for 2.5 h. During this period the reaction mixture became almost colorless. The ethanol was evaporated, and the residue was slowly poured into ice–water. The pale-yellow precipitate was filtered, washed with cold water, dissolved in acetone, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in 100 mL of acetone, and dimethyl sulfate (5.50 g, 4.20 mL, 43.6 mmol) was

added. Then the solution was heated at 60 °C and a KOH solution (3.06 g, 54.5 mmol) in 15 mL of CH_3OH was added dropwise and very slowly (CAUTION: exothermic reaction). Then the mixture was heated under reflux for 3.5 h and afterward stirred overnight at room temperature. The reaction mixture was poured into 300 mL of 5% NaOH solution and stirred for 30 min. Then concentrated HCl was added dropwise until the solution became acidic. The acetone was evaporated and the aqueous residue extracted with diethyl ether. The combined organic layers were washed once with water, dried over $MgSO_4$, and then evaporated. Flash chromatography (SiO_2, petroleum ether/ethyl acetate, 5:2) afforded [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38** (2.98 g, 8.5 mmol, 78%) as a colorless solid. ^{1}H NMR (250 MHz, CD_3OD): δ 8.06 (m, 2H, NqH-5/8), 7.49 (m, 2H, NqH-6/7), 7.15 (d, 2H, PhH), 7.04 (d, 2H, PhH), 4.25 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.53 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 2.20 (s, 3H, CH_3). To [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38** (200 mg, 0.56 mmol, 1 equiv) in 0.2 mL of CH_2Cl_2 was added thionyl chloride (0.2 mL, 2.80 mmol, 5 equiv), and then the reaction mixture was heated to reflux for 1.5 h at 50 °C. After the mixture was cooled at 0 °C, a mixture of *N*-(7-chloroquinolin-4-yl)-*N*-isopropylethyl-1,2-diamine **2** (177.2 mg, 0.70 mmol, 1.2 equiv) and triethylamine (0.16 mL, 1.20 mmol, 2 equiv) in 2 mL of CH_2Cl_2 was added. After the mixture was stirred for 1 h at 0 °C and for a further 12 h at room temperature, the solvent was evaporated and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/CH_3OH, 12:1) to afford *N*-[2-(7-chloroquinolin-4-ylamino)ethyl]-2-[4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]-*N*-isopropylacetamide **40** (205 mg, 0.34 mmol, 61.4%) as a colorless solid, mp 95–97 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.31 (d, 1H, QnH-2, ^3J = 6 Hz), 8.03 (m, 2H, NqH-5/8), 7.91 (d, 1H, QnH-5, J = 9 Hz), 7.77 (d, 1H, QnH-8, J = 2 Hz), 7.46 (m, 2H, NqH-6/7), 7.34 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 7.11 (d, 2H, PhH), 7.00 (d, 2H, PhH), 6.69 (d, 1H, QnH-3, ^3J = 6 Hz), 4.21 (sept, 1H, CH), 4.20 (s, 2H, $COCH_2$), 3.77 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.52 (m, 4H, NCH_2CH_2N), 2.15 (s, 3H, CH_3), 1.04 (d, 6H, ^{i}Pr). ^{13}C NMR (75 MHz, CD_3OD): δ 174.9, 152.9, 152.0, 151.8, 151.7, 148.9, 140.6, 136.6, 133.9, 130.4, 129.8, 129.6, 129.3, 128.6, 128.0, 127.2, 126.9, 126.6, 126.3, 124.2, 123.4, 123.2, 118.4, 99.7, 62.7, 61.7, 50.8, 43.8, 41.7, 40.5, 33.1, 21.1, 12.8. MS (FAB+): 596.8 (M^+ + 1). Anal. ($C_{36}H_{38}ClN_3O_3 \cdot 1H_2O$) C, H, N.

6-(1,4-Dimethoxy-3-methylnaphthalen-2-yl)hexanoic Acid [2-(7-Chloroquinolin-4-ylamino)ethyl]-*N*-isopropylamide 39. 6-(1,4-Dimethoxy-3-methylnaphthalen-2-yl)hexanoic acid **37** was produced using the same protocol as for [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38**. GP5 from [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38** (0.50 g, 1.6 mmol) and *N*′-(7-chloroquinolin-4-yl)-*N*-isopropyl ethane-1,2-diamine **2** (0.51 g, 1.90 mmol) yielded 6-(1,4-dimethoxy-3-methylnaphthalen-2-yl)hexanoic acid [2-(7-chloroquinolin-4-ylamino)ethyl]isopropylamide **39** (63 mg, 0.01 mmol, 7%) as a colorless solid after flash chromatography (SiO_2, CH_2Cl_2/CH_3OH, 20:1 and 1:1) and preparative TLC (SiO_2, CH_2Cl_2/CH_3OH, 12:1), mp 57–59 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.28 (d, 1H, QnH-2, ^3J = 5 Hz), 8.02 (d, 1H, QnH-5, J = 9 Hz), 7.95–7.90 (m, 2H, NqH-5/8), 7.75 (d, 1H, QnH-8, J = 2 Hz), 7.49 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 7.40–7.35 (m, 2H, NqH-6/7), 6.75 (d, 1H, QnH-3, ^3J = 6 Hz), 4.27 (sept, 1H, CH), 3.80 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.57–3.46 (m, 4H, $N_{Qn}CH_2$), 2.75 (m, 2H, CH_2), 2.49 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 1.73–1.62 (m, 2H, CH_2), 1.60–1.44 (m, 4H, CH_2), 1.20 (d, 6H, ^{i}Pr). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.9, 153.8, 150.1, 149.8, 145.4, 142.0, 138.0, 131.4, 127.4, 127.1, 126.9, 126.3, 125.4, 125.3, 123.4, 122.7, 122.2, 116.0, 97.8, 62.1, 61.3, 49.1, 45.6, 39.0, 33.7, 30.0, 29.7, 27.3, 27.1, 25.4, 21.2, 12.3. MS (FAB+): 562.3 (M^+). HRMS (FAB+, NBA): calcd for (M^+ + 1) 562.2836; found 562.2859.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-[4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetamide 41.** GP5 from [4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetic acid **38** (0.50 g, 1.43 mmol, 1 equiv) and *N*-(7-chloroquinolin-4-yl)ethyl-1,2-diamine **1** (0.38 g, 1.72 mmol, 1.2 equiv) yielded *N*-[2-(7-chloroquinolin-4-ylamino)ethyl]-2-[4-(1,4-dimethoxy-3-methylnaphthalen-2-ylmethyl)phenyl]acetamide **41** (116 mg, 0.21 mmol, 14.6%) as a colorless solid, mp 108–110 °C. ^{1}H NMR (250 MHz, CD_3OD): δ 8.30 (d, 1H, QnH-2, ^3J = 5 Hz), 8.04 (m, 2H, NqH-5/8), 7.93 (d, 1H, QnH-5, J = 9 Hz), 7.75 (d, 1H, QnH-8, J = 2 Hz), 7.48 (m, 2H, NqH-6/7), 7.35 (dd, 1H, QnH-6, J = 2 Hz, J = 9 Hz), 7.07 (d, 2H, PhH), 6.95 (d, 2H, PhH), 6.56 (d, 1H, QnH-3, ^3J = 5 Hz), 4.20 (s, 2H, $COCH_2$), 3.78 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.50–3.45 (m, 4H, NCH_2), 3.44 (s, 2H, $PhCH_2$), 2.14 (s, 3H, CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 175.5, 154.5, 151.7, 151.6, 149.1, 145.8, 140.5, 138.0, 134.2, 130.4, 130.1, 129.4, 129.2, 128.5, 128.0, 127.0, 126.8, 126.6, 124.7, 124.6, 123.4, 123.2, 117.8, 99.5, 62.3, 61.7, 44.5, 43.4, 39.1, 33.2, 12.8. MS (FAB+): 554.1 (M^+ + 1). HRMS (FAB+, NBA): calcd (M^+ + 1) 554.2210; found 554.2219.

Assay of β-Hematin Inhibition in Eppendorf Tubes. We determined the IC_{50} values for inhibition of β-hematin formation using Egan's test[45] with very slight modifications as described below. Increasing numbers of equivalents of the 4-aminoquinoline derivatives were prepared using aqueous HCl for CQ and DMF instead of methanol for all other compounds. Hematin stock solution (1.68 mM) was prepared by dissolving bovine hemin in 0.1 M NaOH. The solution was incubated at room temperature for 60 min. In a series of 2 mL Eppendorf tubes 2.0 μL of drug solution (or solvent for the blank) and 2.0 μL of 1 M HCl (when DMF was used, or 2.0 μL of water for CQ) were dispensed. The Eppendorf tubes were placed in an incubator at 60 °C, and then 12.9 M sodium acetate solution, pH 5.0 (11.7 μL) and preincubated at 60 °C, was added. The β-hematin formation process was initiated by addition of hematin stock solution (20.2 μL) prepared above. The final hematin concentration was 1 mM, and the final drug concentrations were 5, 4, 3, 2.5, 2, 1.5, 1, 0.5, and 0 mM for CQ, **2**, and $\mathbf{M_5}$ and were 5,3, 1.5, 1, 0.75, 0.6, 0.5, 0.25, and 0 mM for amide **34**. The pH of the resulting acetate solution was checked to be 4.5 after addition of the hematin solution. The reaction mixtures were incubated at 60 °C for 60 min. Then the reaction mixtures were quenched at room temperature by adding 900 μL of 200 mM HEPES 5% (v/v) pyridine solution, pH 8.2, to adjust the final pH of the mixtures to a value between 7.2 and 7.5. Then 1100 μL of 20 mM HEPES 5% (v/v) pyridine solution, pH 7.5, was added. The Eppendorf tubes were shaken, and the precipitate of β-hematin was scrapped from the walls of the Eppendorf tubes to ensure complete dissolution of hematin. The β-hematin was allowed to settle at room temperature for at least 15 min. The supernatant was carefully transferred to a cuvette without disturbing the precipitate, and absorption was measured at 405 nm.

Parasite Cultures. The CQ-sensitive 3D7 clone of the NF54 isolate[86] of *Plasmodium falciparum* and the chloroquine-, pyrimethamine-, and cycloguanil-resistant K1 strain (Thailand) were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, VA). *P. falciparum* in vitro culture was carried out using standard protocols[87] with modifications. Briefly, parasites were maintained in tissue culture flasks in human A Rh+ erythrocytes at 5% hematocrit in RPMI 1640 supplemented with 25 mM HEPES, 24 mM $NaHCO_3$, 0.2% (w/v) glucose, 0.03% L-glutamine, 150 μM hypoxanthine, and 0.5% Albumax II (Invitrogen) in a 5% CO_2/air mixture at 37 °C, and the medium was changed daily.

In Vitro Antiparasitic Bioassays. Drug susceptibility of *P. falciparum* was studied using a modified method[88] of the protocol described previously.[89] All assays included CQ diphosphate (Sigma, U.K.) as a standard and control wells with untreated infected and uninfected erythrocytes. IC_{50} values were derived by sigmoidal regression analysis (Microsoft *xl*fit). All data from in vitro tests

were carried out in triplicate and were given with the 95% confidence limits.

Effect of Drug Combinations with Glutathione Reductase Inhibitors on *P. falciparum*. Methylene blue was obtained from Roth (Karlsruhe, Germany), CQ and amodiaquine were from Sigma-Aldrich (Steinheim, Germany), mefloquine was from Roche (Mannheim, Germany), and artemisinin was from Aldrich Chem. Co. (Milwaukee, WI). Artemisinin derivatives (artemether and artesunate) and piperaquine tetraphosphate were kindly provided by the Swiss Tropical Institute (Basel, Switzerland) and by Dr. J. Carl Craft, Medicines for Malaria Venture (Geneva, Switzerland), respectively. Isotopic drug sensitivity assays based on the semiautomated microdilution technique[89] were employed to study the effects of selected GR inhibitors in combination with other clinically used antimalarials. The method that depends on the incorporation of radioactive [^{3}H]hypoxanthine, which is taken up by the parasite as a precursor of purine deoxynucleotides for DNA synthesis, was performed according to reported modifications.[90] In 96-well microtiter plates (Nunc), a 2-fold serial dilution of the starting concentration of each drug to be tested was carried out. Two drugs to be tested in combination were applied alone and in fixed concentration ratios of 1:1, 1:3, and 3:1 as described.[90] Parasites were incubated at a parasitemia of 0.125% (>70% ring forms) and 1.25% hematocrit in hypoxanthine-free medium. After 48 h, 0.5 μCi [^{3}H]hypoxanthine was added into each well and the plates were incubated for another 24 h. The cells of each well were harvested on a glass fiber filter (Perkin-Elmer, Rodgau-Jügesheim, Germany), washed, and dried. Their radioactivity in counts per minute was considered to be proportional to the respective growth of *P. falciparum* in the well. IC_{50} values (drug concentrations that produce 50% reduction in the uptake of [^{3}H]hypoxanthine) and IC_{90} values were calculated; the fractional inhibitory concentrations (FIC) of the respective drugs were determined on the basis of the following definitions: $FIC_{50}(A) = [IC_{50}(A + B)]/IC_{50}(A)$; $FIC_{50}(B) = [IC_{50}(B + A)]/IC_{50}(B)$; $FIC_{50} = FIC_{50}(A) + FIC_{50}(B)$.[90]

Evaluation of the Cytotoxicity. Cytotoxicity on KB cells (human oral pharyngeal carcinoma) was evaluated using the Alamar blue assay as described.[88] The positive control drug was podophyllotoxin (Sigma). IC_{50} values were calculated compared to blanks and untreated controls.

In Vivo Antimalarial Activity. Compounds **4**, **21**, and **34** were tested in the *P. berghei* model by using the 4-day suppressive test, as indicated by Peters,[70] and using chloroquine as a positive control. Briefly, naive 18–20 g ANKA BALB/c mice were infected intravenously with 2×10^6 parasitized red cells on day +0. For administration, compounds were freshly prepared in 10% DMSO in sterile phosphate-buffered saline the day of use. Two hours after infection, mice received the first treatment by the intraperitoneal route. Mice were further treated on days +1–3. Blood films from tail blood were prepared on day +4, and parasitemia was determined by microscopic examination of Giemsa-stained blood films. Compounds **4**, **21**, and **34** were tested with a daily dose of 21 mg/kg (**34**) or 30 mg/kg (**4**, **21**) by the intraperitoneal route. Chloroquine treatment po at 10 (mg/kg)/day was included as a positive control and resulted in complete inhibition (data not shown). Intraperitoneous administrations of CQ have shown similar activity (98.9% inhibition at 10 mg/kg ip) in a number of tests but were not done specifically with this series of compounds. Mice were treated and levels of parasitemia determined as described.

Trans Stimulation. The trans-stimulation protocol has been described in full detail previously.[18] Briefly, erythrocytes infected with trophozites of the *P. falciparum* strain Dd2 were resuspended in reaction buffer A (bicarbonate-free RPMI 1640 containing 11 mM glucose and supplemented with 25 mM HEPES-Na and 2 mM glutamine, pH 7.3 at 37 °C) at a hematocrit of 30 000 cells/μL. The hematocrit was determined using a Neubauer counting chamber. The cells were then incubated at 37 °C for 15 min in the presence of different concentrations of either **2** or **34**, ranging from 0 to 10.0 μM. After preloading, cells were washed twice in ice-cold reaction buffer A (pH adjusted to 7.3 at 4 °C) and then resuspended in prewarmed reaction buffer A containing 43 nM [^{3}H]CQ. The mixture was held at 37 °C, and the amount of [^{3}H]CQ accumulation was monitored at 4 min. The intracellular CQ concentration was calculated from the amount of [^{3}H]CQ taken up by the cells and by assuming that the volume of a trophozoite-infected erythrocyte is 75 fL.[91] CQ accumulation was then expressed as the ratio of the intracellular CQ concentration to the extracellular CQ concentration (CQ_{in}/CQ_{out}). The data points were fitted using previously described equations,[18] which, for **2**, assume the presence of a carrier and, for **34**, a simple binding model.

Acknowledgment. Drs. Jens Pfannstiel and Johannes Lechner, Biochemistry Centre, Heidelberg University, are acknowledged for the electrospray-ionization mass spectra of the 4-aminoquinolines derivatives. The excellent technical assistance of Margit Brückner in performing the hematin polymerization assays is gratefully acknowledged. Our work is supported by CNRS-DFG program from the Centre National de la Recherche Scientifique (E.D.-C., W.F.) and by the Deutsche Forschungsgemeinschaft (Project SFB 544 "Control of Tropical Infectious Diseases", Project B14 (E.D.-C., W.F.), Project B12 (M.L., C.P.S.), and Grant BE 1540/4-4 (K.B.)). W.F. is CNRS Junior Fellow from the CNRS-DFG program. This investigation received financial support from the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

Supporting Information Available: X-ray diffraction analysis of the 4-aminoquinoline **7**, including summary of data collection, tables of crystallographical and geometrical data, and elemental analysis results of new compounds **2–5**, **7**, **12–15**, **19–30**, **32**, **34**, **36**, and **40**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chou, A. C.; Chevli, R.; Fitch, C. D. Ferriprotoporphyrin IX fulfills the criteria for identification as the chloroquine receptor of malaria parasites. *Biochemistry* **1980**, *19*, 1543–1549.
(2) Yayon, A.; Cabantchik, Z. I.; Ginsburg, H. Identification of the acidic compartment of *Plasmodium falciparum*-infected human erythrocytes as the target of the antimalarial drug chloroquine. *EMBO J.* **1984**, *3*, 2695–2700.
(3) Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. An assessment of drug−haematin binding as a mechanism for inhibition of haematin polymerisation by quinoline antimalarials. *Biochem. Pharmacol.* **1998**, *55*, 727–736.
(4) Leed, A.; DuBay, K.; Ursos, L. M.; Sears, D.; De Dios, A. C.; Roepe, P. D. Solution structures of antimalarial drug−heme complexes. *Biochemistry* **2002**, *41*, 10245–10255.
(5) De Dios, A. C.; Tycko, R.; Ursos, L. M. B.; Roepe, P. D. NMR studies of chloroquine−ferriprotoporphyrin IX complex. *J. Phys. Chem. A* **2003**, *107*, 5821–5825.
(6) Fitch, C. D. Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. *Life Sci.* **2004**, *74*, 1957–1972.
(7) Fidock, D. A.; Nomura, T.; Talley, A. K.; Cooper, R. A.; Dzekunov, S. M.; Ferdig, M. T.; Ursos, L. M.; Sidhu, A. B.; Naude, B.; Deitsch, K. W.; Su, X. Z.; Wootton, J. C.; Roepe, P. D.; Wellems, T. E. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol. Cell* **2000**, *6*, 861–871.
(8) Djimde, A.; Doumbo, O. K.; Cortese, J. F.; Kayentao, K.; Doumbo, S.; Diourte, Y.; Dicko, A.; Su, X. Z.; Nomura, T.; Fidock, D. A.; Wellems, T. E.; Plowe, C. V.; Coulibaly, D. A molecular marker for chloroquine-resistant falciparum malaria. *N. Engl. J. Med.* **2001**, *344*, 257–263.
(9) Cooper, R. A.; Ferdig, M. T.; Su, X. Z.; Ursos, L. M.; Mu, J.; Nomura, T.; Fujioka, H.; Fidock, D. A.; Roepe, P. D.; Wellems, T. E. Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses in *Plasmodium falciparum*. *Mol. Pharmacol.* **2002**, *61*, 35–42.
(10) Sidhu, A. B.; Verdier-Pinard, D.; Fidock, D. A. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations. *Science* **2002**, *298*, 210–213.
(11) Lakshmanan, V.; Bray, P. G.; Verdier-Pinard, D.; Johnson, D. J.; Horrocks, P.; Muhle, R. A.; Alakpa, G. E.; Hughes, R. H.; Ward,

S. A.; Krogstad, D. J.; Sidhu, A. B.; Fidock, D. A. A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. *EMBO J.* **2005**, *24*, 2294–2305.
(12) Meierjohann, S.; Walter, R. D.; Müller, S. Regulation of intracellular glutathione levels in erythrocytes infected with chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. *Biochem. J.* **2002**, *368*, 761–768.
(13) Martin, R. E.; Kirk, K. The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. *Mol. Biol. Evol.* **2004**, *21*, 1938–1949.
(14) Tran, C. V.; Saier, M. H., Jr. The principal chloroquine resistance protein of *Plasmodium falciparum* is a member of the drug/metabolite transporter superfamily. *Microbiology* **2004**, *150*, 1–3.
(15) Bray, P. G.; Mungthin, M.; Hastings, I. M.; Biagini, G. A.; Saidu, D. K.; Lakshmanan, V.; Johnson, D. J.; Hughes, R. H.; Stocks, P. A.; O'Neill, P. M.; Fidock, D. A.; Warhurst, D. C.; Ward, S. A. PfCRT and the trans-vacuolar proton electrochemical gradient: regulating the access of chloroquine to ferriprotoporphyrin IX. *Mol. Microbiol.* **2006**, *62*, 238–251.
(16) Warhurst, D. C.; Craig, J. C.; Adagu, I. S. Lysosomes and drug resistance in malaria. *Lancet* **2002**, *360*, 1527–1529.
(17) Zhang, H.; Paguio, M.; Roepe, P. D. The antimalarial drug resistance protein *Plasmodium falciparum* chloroquine resistance transporter binds chloroquine. *Biochemistry* **2004**, *43*, 8290–8296.
(18) Sanchez, C. P.; Stein, W.; Lanzer, M. Trans stimulation provides evidence for a drug efflux carrier as the mechanism of chloroquine resistance in *Plasmodium falciparum*. *Biochemistry* **2003**, *42*, 9383–9394.
(19) Sanchez, C. P.; McLean, J. E.; Stein, W.; Lanzer, M. Evidence for a substrate specific and inhibitable drug efflux system in chloroquine resistant *Plasmodium falciparum* strains. *Biochemistry* **2004**, *43*, 16365–16373.
(20) Naude, B.; Brzostowski, J. A.; Kimmel, A. R.; Wellems, T. E. *Dictyostelium discoideum* expresses a malaria chloroquine resistance mechanism upon transfection with mutant, but not wild-type, *Plasmodium falciparum* transporter PfCRT. *J. Biol. Chem.* **2005**, *280*, 25596–25603.
(21) Sanchez, C. P.; McLean, J. E.; Rohrbach, P.; Fidock, D. A.; Stein, W. D.; Lanzer, M. Evidence for a pfcrt-associated chloroquine efflux system in the human malarial parasite *Plasmodium falciparum*. *Biochemistry* **2005**, *44*, 9862–9870.
(22) Sanchez, C. P.; Rohrbach, P.; McLean, J. E.; Fidock, D. A.; Stein, W. D.; Lanzer, M. Differences in trans-stimulated chloroquine efflux kinetics are linked to PfCRT in *Plasmodium falciparum*. *Mol. Microbiol.* **2007**, *64*, 407–420.
(23) De, D.; Krogstad, F. M.; Cogswell, F. B.; Krogstad, D. J. Aminoquinolines that circumvent resistance in *Plasmodium falciparum* in vitro. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 579–583.
(24) De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. Structure−activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. *J. Med. Chem.* **1998**, *41*, 4918–4926.
(25) Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. Novel short chain chloroquine analogues retain activity against chloroquine resistant K1 *Plasmodium falciparum*. *J. Med. Chem.* **2002**, *45*, 4975–4983.
(26) Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M. A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. 4-Aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846–1854.
(27) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S. Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene-chloroquine analogue. *J. Med. Chem.* **1997**, *40*, 3715–3718.
(28) Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. Structure−function relationships in aminoquinolines: effect of amino and chloro groups on quinoline−hematin complex formation, inhibition of beta-hematin formation, and antiplasmodial activity. *J. Med. Chem.* **2000**, *43*, 283–291.
(29) Solomon, V. R.; Puri, S. K.; Srivastava, K.; Katti, S. B. Design and synthesis of new antimalarial agents from 4-aminoquinoline. *Bioorg. Med. Chem.* **2005**, *13*, 2157–2165.
(30) Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D. Structure−activity relationships in 4-aminoquinoline antiplasmodials. The role of the group at the 7-position. *J. Med. Chem.* **2002**, *45*, 3531–3539.
(31) Madrid, P. B.; Sherrill, J.; Liou, A. P.; Weisman, J. L.; Derisi, J. L.; Guy, R. K. Synthesis of ring-substituted 4-aminoquinolines and evaluation of their antimalarial activities. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1015–1018.
(32) Vippagunta, S. R.; Dorn, A.; Matile, H.; Bhattacharjee, A. K.; Karle, J. M.; Ellis, W. Y.; Ridley, R. G.; Vennerstrom, J. L. Structural specificity of chloroquine−hematin binding related to inhibition of hematin polymerization and parasite growth. *J. Med. Chem.* **1999**, *42*, 4630–4639.
(33) Cheruku, S. R.; Maiti, S.; Dorn, A.; Scorneaux, B.; Bhattacharjee, A. K.; Ellis, W. Y.; Vennerstrom, J. L. Carbon isosteres of the 4-aminopyridine substructure of chloroquine: effects on pK(a), hematin binding, inhibition of hemozoin formation, and parasite growth. *J. Med. Chem.* **2003**, *46*, 3166–3169.
(34) Madrid, P. B.; Wilson, N. T.; DeRisi, J. L.; Guy, R. K. Parallel synthesis and antimalarial screening of a 4-aminoquinoline library. *J. Comb. Chem.* **2004**, *6*, 437–442.
(35) Madrid, P. B.; Liou, A. P.; DeRisi, J. L.; Guy, R. K. Incorporation of an intramolecular hydrogen-bonding motif in the side chain of 4-aminoquinolines enhances activity against drug-resistant *P. falciparum*. *J. Med. Chem.* **2006**, *49*, 4535–4543.
(36) Zhang, Y.; König, I.; Schirmer, R. H. Glutathione reductase-deficient erythrocytes as host cells of malarial parasites. *Biochem. Pharmacol.* **1988**, *37*, 861–865.
(37) Zhang, Y. A.; Hempelmann, E.; Schirmer, R. H. Glutathione reductase inhibitors as potential antimalarial drugs. Effects of nitrosoureas on *Plasmodium falciparum* in vitro. *Biochem. Pharmacol.* **1988**, *37*, 855–860.
(38) Florence, T. M. The degradation of cytochrome c by hydrogen peroxide. *J. Inorg. Biochem.* **1985**, *23*, 131–141.
(39) Garel, M. C.; Domenget, C.; Caburi-Martin, J.; Prehu, C.; Galacteros, F.; Beuzard, Y. Covalent binding of glutathione to hemoglobin. I. Inhibition of hemoglobin S polymerization. *J. Biol. Chem.* **1986**, *261*, 14704–14709.
(40) Craescu, C. T.; Poyart, C.; Schaeffer, C.; Garel, M. C.; Kister, J.; Beuzard, Y. Covalent binding of glutathione to hemoglobin. II. Functional consequences and structural changes reflected in NMR spectra. *J. Biol. Chem.* **1986**, *261*, 14710–14716.
(41) Wodak, S. J.; De Coen, J. L.; Edelstein, S. J.; Demarne, H.; Beuzard, Y. Modification of human hemoglobin by glutathione. III. Perturbations of hemoglobin conformation analyzed by computer modeling. *J. Biol. Chem.* **1986**, *261*, 14717–14724.
(42) Atamna, H.; Ginsburg, H. Heme degradation in the presence of glutathione. A proposed mechanism to account for the high levels of non-heme iron found in the membranes of hemoglobinopathic red blood cells. *J. Biol. Chem.* **1995**, *270*, 24876–24883.
(43) Ginsburg, H.; Famin, O.; Zhang, J.; Krugliak, M. Inhibition of glutathione-dependent degradation of heme by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. *Biochem. Pharmacol.* **1998**, *56*, 1305–1313.
(44) Steele, J. C.; Phelps, R. J.; Simmonds, M. S.; Warhurst, D. C.; Meyer, D. J. Two novel assays for the detection of haemin-binding properties of antimalarials evaluated with compounds isolated from medicinal plants. *J. Antimicrob. Chemother.* **2002**, *50*, 25–31.
(45) Ncokazi, K. K.; Egan, T. J. A colorimetric high-throughput beta-hematin inhibition screening assay for use in the search for antimalarial compounds. *Anal. Biochem.* **2005**, *338*, 306–319.
(46) Krauth-Siegel, R. L.; Bauer, H.; Schirmer, R. H. Dithiol proteins as guardians of the intracellular redox milieu in parasites: old and new drug targets in trypanosomes and malaria-causing plasmodia. *Angew. Chem., Int. Ed.* **2005**, *44*, 690–715.
(47) Dubois, V. L.; Platel, D. F.; Pauly, G.; Tribouley-Duret, J. *Plasmodium berghei*: implication of intracellular glutathione and its related enzyme in chloroquine resistance in vivo. *Exp. Parasitol.* **1995**, *81*, 117–124.
(48) Davioud-Charvet, E.; Delarue, S.; Biot, C.; Schwöbel, B.; Böhme, C. C.; Müssigbrodt, A.; Maes, L.; Sergheraert, C.; Grellier, P.; Schirmer, R. H.; Becker, K. A prodrug form of a *Plasmodium falciparum* glutathione reductase inhibitor conjugated with a 4-anilinoquinoline. *J. Med. Chem.* **2001**, *44*, 4268–4276.
(49) Biot, C.; Bauer, H.; Schirmer, R. H.; Davioud-Charvet, E. 5-Substituted tetrazoles as bioisosters of carboxylic acids. Bioisosterism and mechanistic studies on glutathione reductase inhibitors as antimalarials. *J. Med. Chem.* **2004**, *47*, 5972–5983.
(50) Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives. *Bioorg. Med. Chem.* **2005**, *13*, 3249–3261.
(51) Singh, C.; Malik, H.; Puri, S. K. Synthesis and antimalarial activity of a new series of trioxaquines. *Bioorg. Med. Chem.* **2004**, *12*, 1177–1182.
(52) Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B. Preparation and antimalarial activities of "trioxaquines", new modular molecules with a trioxane skeleton linked to a 4-aminoquinoline. *ChemBioChem* **2000**, *1*, 281–283.
(53) Beagley, P.; Blackie, M. A. L.; Chibale, K.; Clarkson, C.; Meijboom, R.; Moss, J. R.; Smith, P. J.; Su, H. Synthesis and antiplasmodial activity in vitro of new ferrocene−chloroquine analogues. *Dalton Trans.* **2003**, *15*, 3046–3051.

(54) Flipo, M.; Florent, I.; Grellier, P.; Sergheraert, C.; Deprez-Poulain, R. Design, synthesis and antimalarial activity of novel, quinoline-based, zinc metallo-aminopeptidase inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2659–2662.
(55) Romeo, S.; Dell'Agli, M.; Parapini, S.; Rizzi, L.; Galli, G.; Mondani, M.; Sparatore, A.; Taramelli, D.; Bosisio, E. Plasmepsin II inhibition and antiplasmodial activity of primaquine-statine "double-drugs". *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2931–2934.
(56) Bernadou, J.; Meunier, B. Biomimetic chemical catalysts in the oxidative activation of drugs. *Adv. Synth. Catal.* **2004**, *346*, 171–184.
(57) Bauer, H.; Fritz-Wolf, K.; Winzer, A.; Kuhner, S.; Little, S.; Yardley, V.; Vezin, H.; Palfey, B.; Schirmer, R. H.; Davioud-Charvet, E. A fluoro analogue of the menadione derivative 6-[2′-(3′-methyl)-1′,4′-naphthoquinolyl]hexanoic acid is a suicide substrate of glutathione reductase. Crystal structure of the alkylated human enzyme. *J. Am. Chem. Soc.* **2006**, *128*, 10784–10794.
(58) Kumpaty, H. J.; Bhattacharyya, S.; Rehr, E. W.; Gonzalez, A. M. Selective access to secondary amines by a highly controlled reductive mono-N-alkylation of primary amines. *Synthesis* **2003**, *14*, 2206–2210.
(59) Assef, G.; Kister, J.; Metzger, J. Synthesis and study of the SR.dblarw. NR rearrangement of 1,3-diazoles and 1,3-diazines. 1-Alkyl-2-(methylthio)-D2-imidazolines and 1-alkyl-2-(methylthio)-D2-tetrahydropyrimidines. *Bull. Soc. Chim. Fr.* **1979**, *3–4* (Part 2), 165–176.
(60) Peck, R. M.; Preston, R. K.; Creech, H. J. Mono- and difunctional analogs of some quinoline and acridine nitrogen mustards. *J. Org. Chem.* **1961**, *26*, 3409–3414.
(61) Bjorkman, S.; Castensson, S.; Sievertsson, H. Tripeptide analogues of melanocyte-stimulating hormone release-inhibiting hormone (Pro-Leu-Gly-NH_2) as inhibitors of oxotremorine-induced tremor. *J. Med. Chem.* **1979**, *22*, 931–935.
(62) Koenig, S.; Muller, L.; Smith, D. K. Dendritic biomimicry: microenvironmental hydrogen-bonding effects on tryptophan fluorescence. *Chemistry* **2001**, *7*, 979–986.
(63) Van Tuyen, N.; Kesteleyn, B.; De Kimpe, N. Synthesis of 2-alkoxymethyl-3-fluoromethyl-1,4-naphthoquinones. *Tetrahedron* **2002**, *58*, 121–127.
(64) Hünig, S.; Bau, R.; Kemmer, M.; Meixner, H.; Metzenthin, T.; Peters, K.; Sinzger, K.; Gulbis, J. Multistep reversible redox systems. Part 63. 2,5-Disubstituted *N*,*N*′-dicyanoquinonediimines (DCNQIs). Syntheses and redox properties. *Eur. J. Org. Chem.* **1998**, 335–348.
(65) Dueno, E. E.; Chu, F.; Kim, S.-I.; Jung, K. W. Cesium promoted O-alkylation of alcohols for efficient ether synthesis. *Tetrahedron Lett.* **1999**, *40*, 1843–1846.
(66) Evans, P. A.; Brandt, T. A. Hypervalent iodine chemistry. Mechanistic investigation of the novel haloacetoxylation, halogenation, and acetoxylation reactions of 1,4-dimethoxynaphthalenes. *J. Org. Chem.* **1997**, *62*, 5321–5326.
(67) Bhattacharyya, S.; Chatterjee, A.; Williamson, J. S. An efficient, safe and convenient one-step synthesis of β-phenethylamines via reductive amination reactions utilizing $Ti(OCHMe_2)_4$ and $NaBH_4$. *Synlett* **1995**, 1079–1080.
(68) Salmon-Chemin, L.; Buisine, E.; Yardley, V.; Kohler, S.; Debreu, M. A.; Landry, V.; Sergheraert, C.; Croft, S. L.; Krauth-Siegel, R. L.; Davioud-Charvet, E. 2- and 3-substituted 1,4-naphthoquinone derivatives as subversive substrates of trypanothione reductase and lipoamide dehydrogenase from *Trypanosoma cruzi*: synthesis and correlation between redox cycling activities and in vitro cytotoxicity. *J. Med. Chem.* **2001**, *44*, 548–565.
(69) de Villiers, K. A.; Kaschula, C. H.; Egan, T. J.; Marques, H. M. Speciation and structure of ferriprotoporphyrin IX in aqueous solution: spectroscopic and diffusion measurements demonstrate dimerization, but not mu-oxo dimer formation. *J. Biol. Inorg. Chem.* **2007**, *12*, 101–117.
(70) Peters, W.; Robinson, B. L. *Handbook of Animal Models of Infection*; Academic Press: London, 1999; pp 756–771.
(71) Sanchez, C. P.; Stein, W. D.; Lanzer, M. Is PfCRT a channel or a carrier? Two competing models explaining chloroquine resistance in *Plasmodium falciparum*. *Trends Parasitol.* **2007**, *23*, 332–339.
(72) Musonda, C. C.; Taylor, D.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. Application of multi-component reactions to antimalarial drug discovery. Part 1: Parallel synthesis and antiplasmodial activity of new 4-aminoquinoline Ugi adducts. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3901–3905.
(73) Solomon, V. R.; Haq, W.; Srivastava, K.; Puri, S. K.; Katti, S. B. Synthesis and antimalarial activity of side chain modified 4-aminoquinoline derivatives. *J. Med. Chem.* **2007**, *50*, 394–398.
(74) Johnson, D. J.; Fidock, D. A.; Mungthin, M.; Lakshmanan, V.; Sidhu, A. B.; Bray, P. G.; Ward, S. A. Evidence for a central role for PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents. *Mol. Cell* **2004**, *15*, 867–877.
(75) Constantino, L.; Iley, J. Oxidation of tertiary benzamides by 5,10,15,20-tetraphenylporphyrinatoironIII chloride-*tert*-butylhydroperoxide. *Org. Biomol. Chem.* **2004**, *2*, 1894–1900.
(76) Niklas, N.; Heinemann, F. W.; Hampel, F.; Clark, T.; Alsfasser, R. The activation of tertiary carboxamides in metal complexes: an experimental and theoretical study on the methanolysis of acylated bispicolylamine copper(II) complexes. *Inorg. Chem.* **2004**, *43*, 4663–4673.
(77) Niklas, N.; Hampel, F.; Liehr, G.; Zahl, A.; Alsfasser, R. The reactivity of N-coordinated amides in metallopeptide frameworks: molecular events in metal-induced pathogenic pathways. *Chemistry* **2001**, *7*, 5135–5142.
(78) Roberts, E. S.; Vaz, A. D.; Coon, M. J. Catalysis by cytochrome P-450 of an oxidative reaction in xenobiotic aldehyde metabolism: deformylation with olefin formation. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8963–8966.
(79) Cornet, M.; Rogiers, V. Metabolism and toxicity of 2-methylpropene (isobutene)—a review. *Crit. Rev. Toxicol.* **1997**, *27*, 223–232.
(80) Pearson, D. E.; Jones, W. H.; Cope, A. C. Synthesis of monoalkyl-substituted diamines and their condensation products with 4,7-dichloroquinoline. *J. Am. Chem. Soc.* **1946**, *68*, 1225–1229.
(81) Elderfield, R. C.; Gensler, W. J.; Birstein, O.; Kreysa, F. J.; Maynard, J. T.; Galbreath, J. Synthesis of certain simple 4-aminoquinoline derivatives. *J. Am. Chem. Soc.* **1946**, *68*, 1250–1251.
(82) Lettieri, G.; Brancaccio, G.; Larizza, A. Synthesis and pharmacological activity of 4-((7-chloro-quinolyl)amino)acylamides. *Boll. Chim. Farm.* **1981**, *120*, 308–310.
(83) Aldersley, M. F.; Dean, F. M.; Mann, B. E. Adducts from quinones and diazoalkanes. Part 10. 2-Diazopropane and 2-methyl-1,4-naphthoquinone; structures and conformations of novel vinylic quinones and epoxides. *J. Chem. Soc., Perkin Trans. 1* **1986**, *12*, 2217–2222.
(84) Harvey, R. G.; Dai, Q.; Ran, C.; Penning, T. M. Synthesis of the *o*-quinones and other oxidized metabolites of polycyclic aromatic hydrocarbons implicated in carcinogenesis. *J. Org. Chem.* **2004**, *69*, 2024–2032.
(85) Ito, T.; Ikemoto, T.; Yamano, T.; Mizuno, Y.; Tomimatsu, K. Practical synthesis of (*R*)-(+)-6-(1,4-dimethoxy-3-methyl-2-naphthyl)-6-(4-hydroxyphenyl)hexanoic acid: a key intermediate for a therapeutic drug for neurodegenerative diseases. *Tetrahedron: Asymmetry* **2003**, *14*, 3525–3531.
(86) Ponnudurai, T.; Leeuwenberg, A. D.; Meuwissen, J. H. Chloroquine sensitivity of isolates of *Plasmodium falciparum* adapted to in vitro culture. *Trop. Geogr. Med.* **1981**, *33*, 50–54.
(87) Trager, W.; Jensen, J. B. Human malaria parasites in continuous culture. *Science* **1976**, *193*, 673–675.
(88) Cameron, A.; Read, J.; Tranter, R.; Winter, V. J.; Sessions, R. B.; Brady, R. L.; Vivas, L.; Easton, A.; Kendrick, H.; Croft, S. L.; Barros, D.; Lavandera, J. L.; Martin, J. J.; Risco, F.; Garcia-Ochoa, S.; Gamo, F. J.; Sanz, L.; Leon, L.; Ruiz, J. R.; Gabarro, R.; Mallo, A.; Gomez de las Heras, F. Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed anti-malarial activity. *J. Biol. Chem.* **2004**, *279*, 31429–31439.
(89) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(90) Fivelman, Q. L.; Adagu, I. S.; Warhurst, D. C. Modified fixed-ratio isobologram method for studying in vitro interactions between atovaquone and proguanil or dihydroartemisinin against drug-resistant strains of *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **2004**, *48*, 4097–4102.
(91) Saliba, K. J.; Horner, H. A.; Kirk, K. Transport and metabolism of the essential vitamin pantothenic acid in human erythrocytes infected with the malaria parasite *Plasmodium falciparum*. *J. Biol. Chem.* **1998**, *273*, 10190–10195.

JM7009292