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Replacement of the 4′-Hydroxy Group of Amodiaquine and Amopyroquine by Aromatic and Aliphatic Substituents: Synthesis and Antimalarial Activity

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The prophylactic administration of amodiaquine (AQ), a 4-aminoquinoline antimalarial drug, has been associated with side effects such as agranulocytosis and liver damage. The toxicity of this drug is mediated by amodiaquine quinone-imine, an electrophilic metabolite. Replacement of the 4′-hydroxy function of AQ with various alkyl, aryl, or heteroaryl substituents would provide analogues that avoid metabolism to potentially toxic derivatives. Following a multistep procedure, 33 compounds containing hydrophobic groups at the 4′-position were synthesized using Csp^2–Csp^2 and Csp^2–Csp^3 Suzuki–Miyaura cross-coupling reactions as the key step. The new derivatives were found to be active against both chloroquine (CQ)-sensitive and CQ-resistant strains of *P. falciparum*, with IC_{50} values in the range of 7–200 nM. Alkyl analogues are more efficient than aryl or heteroaryl derivatives. All compounds were also assessed for their cytotoxicity and ability to inhibit β-hematin formation in vitro. A detailed investigation of the structure–activity relationships for these new compounds was carried out; the 4′-methyl compound showed interesting in vivo antimalarial activity.

Introduction

It has been estimated that malaria affects more than 2.4 billion people in more than 100 countries, accounting for approximately 40% of the world's population.[1] Despite considerable progress in the control of this disease over the past 60 years, it remains a serious health problem—particularly in sub-Saharan Africa, where about 90% of the clinical cases occur.[2] The widespread emergence of parasite resistance to currently available antimalarials, notably to chloroquine (CQ, Figure 1), has caused a re-evaluation of alternative drugs to circumvent this problem.

The process of hemoglobin degradation inside the parasite food vacuole and the interaction with the host-dependent heme molecule remain promising areas for antimalaria drug discovery.[3] 4-Aminoquinoline drugs (such as CQ, amodiaquine (AQ), and amopyroquine (ApQ); Figure 1) are concentrated in the parasite food vacuole and are thought to act by preventing or disrupting the effective formation of hemozoin, resulting in heme-mediated toxicity to the parasite.[4] 4-Aminoquinoline resistance spreads relatively slowly and has been associated mainly with mutations in genes of transporter proteins, such as pfCRT,[5] which affect drug access or extrusion in the parasite food vacuole. Thus, it can be expected that relatively small structural changes could enhance drug accumulation in resistant parasites.

AQ (Figure 1), developed in the early 1950s,[6] proved to be effective against many CQ-resistant strains.[7] However, the use of this drug was limited in the mid-1980s, as it was associated with some cases of agranulocytosis and hepatotoxicity in prophylaxis.[8,9] Subsequent detailed investigations have shown

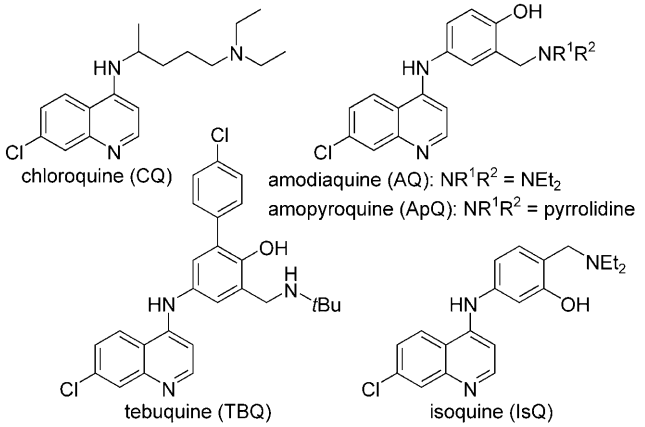

Figure 1. Structures of some 4-aminoquinoline antimalarials.

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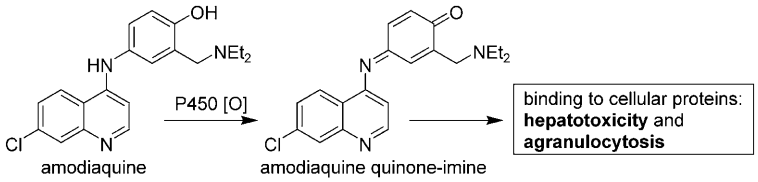
that AQ is no more toxic than CQ when used therapeutically to treat uncomplicated *Plasmodium falciparum* malaria. Its high efficacy against many CQ-resistant strains spurred a recent increase in its re-evaluation and use.[7c,10] Although resistance to AQ is also developing,[11] the World Health Organization (WHO) guidelines for treatment still recommend its use in combination with artemisinin derivatives or, if not available, with sulfadoxine/pyrimethamine. The combination of artesunate and amodiaquine has been marketed under the trade name Arsucam since 2007.[10c,d,12]



Scheme 1. Oxidation of amodiaquine to the toxic quinone-imine electrophilic metabolite mediated by cytochrome P450.

Relative to CQ, AQ exhibits greater parasite-specific accumulation and activity and a markedly decreased cross-resistance pattern in vitro.[13] The main difference between the two drugs is the presence of a *p*-hydroxyaniline aromatic unit that influences not only the conformational flexibility of the side chain but also the physicochemical properties such as basicity or lipophilicity. Whether or not the 4′-hydroxy group is important for antimalarial activity against CQ-resistant strains remains controversial. Previous studies suggested that the relative position of the 4′-hydroxy group and the amine in the side chain may favor an "active structure" stabilized by an intramolecular hydrogen bond,[4a,b,13a] which has been associated with the high intravacuolar accumulation of this drug.

In vivo, AQ is extensively transformed into its primary metabolite, desethylAQ (DesAQ), which, unlike AQ, presents cross-resistance with CQ.[14] ApQ (Figure 1), a structural analogue of AQ in which the diethylamino side chain is replaced by a pyrrolidinyl cycle, showed improved metabolic stability[14–16] and proved to be more active than AQ and DesAQ against CQ-resistant strains.[14,15]

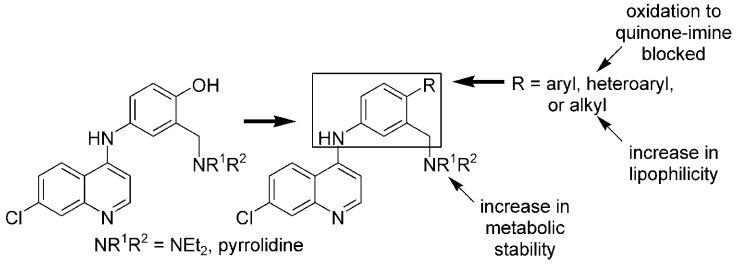
The development of 5′-alkyl-substituted AQ analogues with a *tert*-butyl group in the Mannich side chain[17] revealed that the increase in drug lipophilicity may also be correlated with a decrease in cross-resistance to CQ. Similar derivatives, in which the 5′-position is substituted with various aromatic functionalities, underscore the importance of hydrophobic groups, which have been associated with an increase in antimalarial activity against CQ-resistant strains and also an extended half-life.[18] The small size and increased electron-withdrawing properties of the phenyl ring substituents were found to be favorable for antimalarial potency. Optimal activity has been observed with tebuquine (TBQ), which has a 4-chlorophenyl group at the 5′-position and a *tert*-butyl substitution in the side chain (Figure 1). TBQ has better antimalarial activity than AQ both in vivo[18] and in vitro.[4a,b] However, further investigation of this compound revealed toxicity toward neutrophils in animals.[9a,d,19]

Although the *p*-hydroxyaniline moiety is associated with the high antimalarial activity of AQ, it is also considered responsible for the observed side effects. This unit may undergo oxidation, leading to AQ quinone-imine (AQQI) (Scheme 1), an electrophilic metabolite.[9] Subsequent oxidative stress or reactions between AQQI and cysteine thiol groups (e.g., glutathione or essential proteins), leading to the formation of 5′-thioethers, are associated with AQ-mediated toxicity.[20]

Significant efforts have been directed toward the development of AQ analogues that avoid metabolism to potentially toxic compounds. One strategy is the introduction of various side chains at the 5′-position, including a second Mannich amino functionality (cycloquine),[21] alkyl,[17] and aryl[4a,b,18] groups, thus avoiding the formation of 5′-thioethers. Another strategy has led to the synthesis of isoquine-like analogues (Figure 1) with good antimalarial activities against CQ-resistant strains.[20d] In these regioisomers the interchange of the 4′-hydroxy group with the Mannich side chain prevents oxidation to toxic metabolites, while the structure retains the potential for intramolecular hydrogen bonding interactions.

In a continuation of our previous studies on the development of new AQ analogues that do not have the 4′-hydroxy group structural liability[22] we were interested in identifying the mechanism responsible for the antimalarial activity of 4′-dehydroxy-AQ derivatives. Our strategy involves the replacement of the 4′-hydroxy function of AQ with various alkyl, aryl, or heteroaryl substituents (Scheme 2). The proposed structural modification allows not only an increase in lipophilicity (similar to that of AQ 5′-alkyl- and aryl-substituted derivatives) but also hinders in vivo oxidation of the 4′-position in quinone-imine intermediates and challenges the presence of the 4′-hydroxy group as necessary for antimalarial activity against CQ-resistant *P. falciparum* strains.

In the case of the 4′-alkyl analogues (Scheme 2), structural diversity implied the introduction of various chain lengths. For the 4′-aryl analogues, phenyl ring derivatization was studied in detail to optimize antimalarial activity and was designed to introduce systematic changes to the structural and physicochemical properties (pK_a and $\log D$) of the compounds. The synthetic strategy and some of the compounds discussed herein have been described by us previously.[23] Correlations



Scheme 2. Design of amodiaquine and amopyroquine analogues.

between the antimalarial activity, cytotoxicity, and the position (*ortho*, *meta*, *para*), nature, and electronic properties of the phenyl ring substituents were foreseen. The effect of replacing the aryl moiety with heteroaromatic cycles (such as furan or thiophene) was also studied. We were also interested in comparing the differences between alkyl-, aryl-, and heteroaryl-substituted derivatives. The introduction of an aromatic ring introduces not only the possibility of supplementary π–π stacking interactions with the supposed target (the ferriprotoporphyrin IX unit), but also important structural rigidity with the formation of the biphenyl unit, relative to simple hydrocarbon chains. We completed the SAR study with the development of a parallel series of ApQ derivatives (Scheme 2).

Results and Discussion

Chemistry

The main criteria for the design and synthesis of these compounds were low-cost preparation, and effective and simple synthetic strategies that allow access to both AQ and ApQ analogues. Unsubstituted compounds, deOH-AQ and deOH-ApQ used as references, were synthesized according to published procedures by starting from commercially available 1-bromomethyl-3-nitrobenzene.[22b]

The 4′-aryl, heteroaryl-, and alkyl-substituted AQ analogues **10a**–**26a** and ApQ analogues **10b**–**26b** were obtained by following a six-step procedure, starting from commercially available 4-nitrobromobenzene (Scheme 3). The study and synthesis of some compounds (derivatives **10a,b**, **11a,b**, **13a,b**–**17a,b**, **20a,b**–**22a,b**, and **24a,b**–**26a,b**) have been presented previously.[23] The series has now been completed with the synthesis of other representative compounds of the series: **12a,b**, **18a,b**, **19a,b**, and **23a,b**.

Suzuki–Miyaura cross-coupling intermediates **2a**–**5a** and **2b**–**5b** were obtained with moderate to good yield. In the case of the 4′-*n*-butyl-substituted derivatives **2a,b**, the experimental conditions were modified from the described procedures for alkyl derivatives.[23,24] Intermediates **2a**–**5a** and **2b**–**5b** were then subjected to reduction of the nitro group. Regioselective substitution of the chlorine atom at the 4-position of 4,7-dichloroquinoline with aniline derivatives **6a**–**9a** and **6b**–**9b** provided AQ analogues **12a**, **19a**, **20a**, and **23a**, and ApQ analogues **12b**, **19b**, **20b**, and **23b**.

Among all the alkyl, aryl, and heteroaryl derivatives, three (**11b**, **20b**, and **22a**) were successfully crystallized, and solid-state structures were determined by X-ray diffractometry (see Figures 2, 3, and 4).[25] Compound **11b** crystallizes with a methanol molecule, which was used as solvent (Figure 2). The torsion angle values of compound **20b** in the solid state indicates that the newly introduced benzene ring and the quinoline ring system are nearly coplanar (Figure 3). In the case of compound **22a** the steric hindrance introduced by the presence of the fluorine atom at the *ortho* position leads to an increase in the torsion angle that corresponds to the biphenyl system (Figure 4).

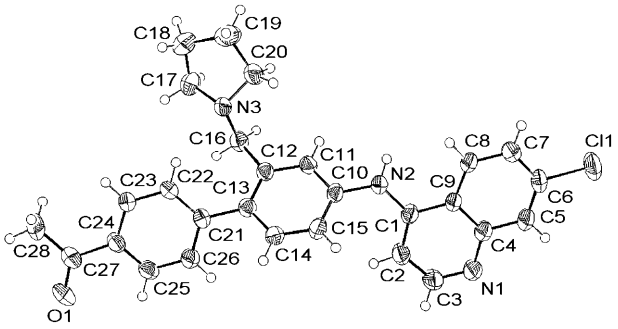


Figure 2. X-ray crystal structure of compound **20b**.

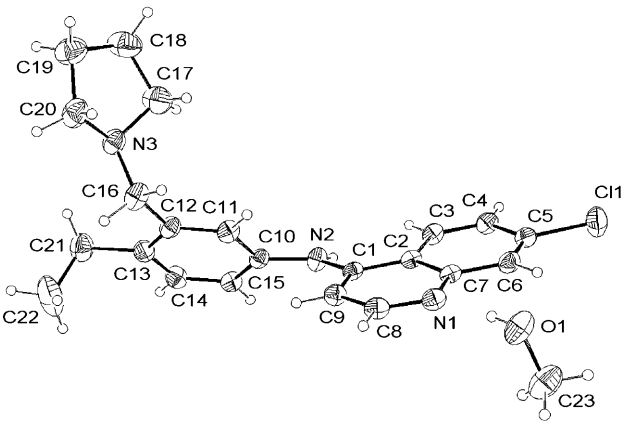
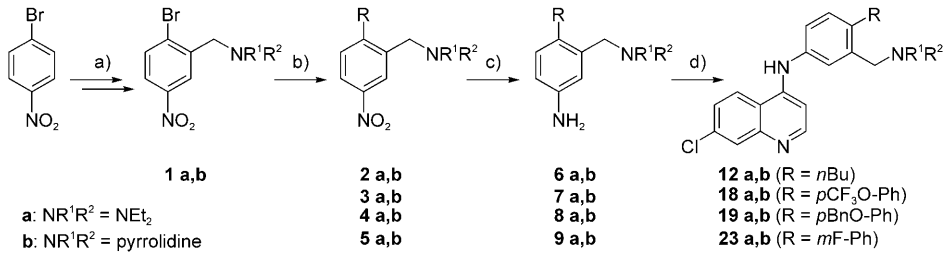


Figure 3. X-ray crystal structure of compound **11b**.



Scheme 3. Synthesis of compounds **12a,b**, **18a,b**, **19a,b**, and **23a,b** via a Suzuki–Miyaura cross-coupling reaction as the key step; reagents and conditions: a) reference [22]; b) R-B(OH)$_2$, Pd(OAc)$_2$, P(*o*-tol)$_3$, and TBAB, Na$_2$CO$_3$, toluene, EtOH, 60 °C for aryl boronic acids, and K$_2$CO$_3$, THF, H$_2$O, 75 °C for alkyl boronic acids; c) SnCl$_2$, HCl, THF, reflux; d) 4,7-dichloroquinoline, HCl, CH$_3$CN, reflux.

Biological results

All compounds were tested against two CQ-sensitive strains (F32 and Thai) and three CQ-re-

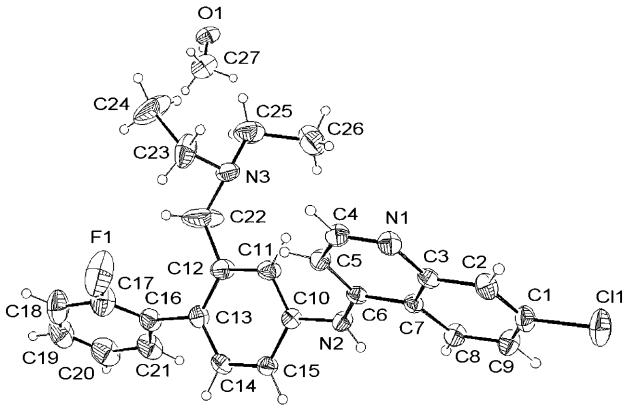


Figure 4. X-ray crystal structure of compound **22a**.

sistant strains (PFB, FcB1R, and K1) of *P. falciparum* (Figures 5 and 6, and Table 1). As IC_{50} values could lead to erroneous conclusions about the efficacy of the compounds, IC_{90} values were also calculated and discussed for the F32 and K1 strains (Table 1). In parallel, the cytotoxicity of the various compounds was evaluated with human MRC-5 cells (diploid embryonic lung cell line; Table 1). The derivatives were also evaluated for their ability to inhibit β-hematin formation, the commonly accepted mechanism of action of 4-aminoquinolines (Figures 5 and 6).

IC_{50} values for AQ and ApQ were found to be quite consistent across the strains tested, regardless of CQ-resistance status, with ApQ showing greater activity (IC_{50} values in the range of 4–5 nM; Table 1, Figure 5). As already described,[22b] 4′-deOH-AQ showed a slight decrease in activity relative to AQ. The same observation was made for 4′-deOH-ApQ and ApQ.

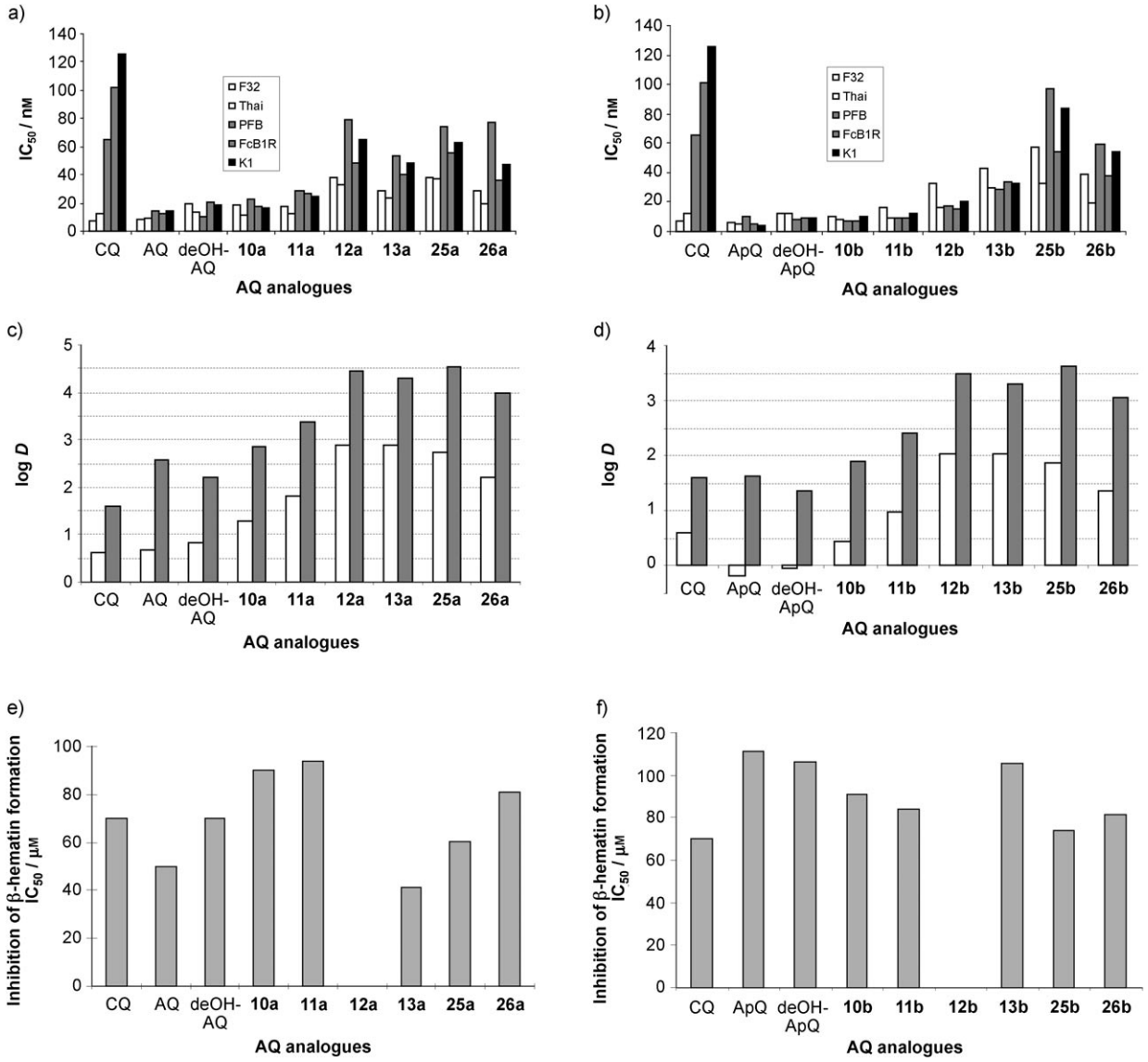


Figure 5. Comparison between in vitro antimalarial activity, lipophilicity, and in vitro inhibition of β-hematin formation of alkyl, phenyl, and heteroaryl compounds. a), b): Efficiency (IC_{50}) of a) AQ and b) ApQ analogues to inhibit the growth of parasites expressing varying degrees of resistance to CQ (indicated). c), d): Calculated log *D* at pH 5.0 (white bars) and pH 7.4 (gray bars) of c) AQ and d) ApQ analogues. e), f): In vitro inhibition of β-hematin formation of e) AQ and f) ApQ analogues; (not determined for compound **12a**).

Except for compounds **15a** and **18a**, all compounds were more efficient than CQ in inhibiting the CQ-resistant strains. Furthermore, most of the compounds showed similar IC_{50} values toward all strains tested, with the exception of the phenyl-substituted compounds **15a**, **16a**, **18a**, **19a**, and **19b**. These hydrophobic analogues proved to be less active against the F32 CQ-sensitive strain, which is also resistant to mefloquine, than they are against the CQ-resistant strains. In general, ApQ analogues **10b**–**26b** showed better antimalarial activity than AQ analogues **10a**–**26a**.

Methyl- and ethyl-substituted compounds **10a**, **10b**, and **11b** were the most potent derivatives of both series, with IC_{50} values in the same range as AQ or deOH-AQ (Figure 5, Table 1). No marked difference in IC_{50} values was observed in correlation to the CQ-resistance status of the strains tested. Increasing the size of the alkyl chain or replacing it with a phenyl group provided less active compounds. The same observations were made for the IC_{90} values of alkyl analogues toward the CQ-resistant strain K1; they remain less than 50 nM, except for compound **12a** (Table 1). Surprisingly, the butyl-AQ analogue **12a** was less efficient than the phenyl-AQ analogue **13a** in blocking parasite growth, whereas the opposite was observed for the ApQ analogues. The decrease in activity, correlated with hydrophobicity and bulkiness, was even more significant for the heteroaryl compounds (Figure 5) or substituted compounds (Figure 6).

In the aryl series, substitution of the phenyl ring with several substituents did not influence antiplasmodial activity (Figure 6). This was the case for the AQ analogues substituted at the *para* position with methoxy (**17a**) or acetyl (**20a**) groups toward all strains, and for compounds substituted with fluoro (**24a**) or benzyloxy (**19a**) groups toward the CQ-resistant strain K1. Those compounds provided about the same activity, with IC_{50} values ~30–40 nM. Similar values were obtained

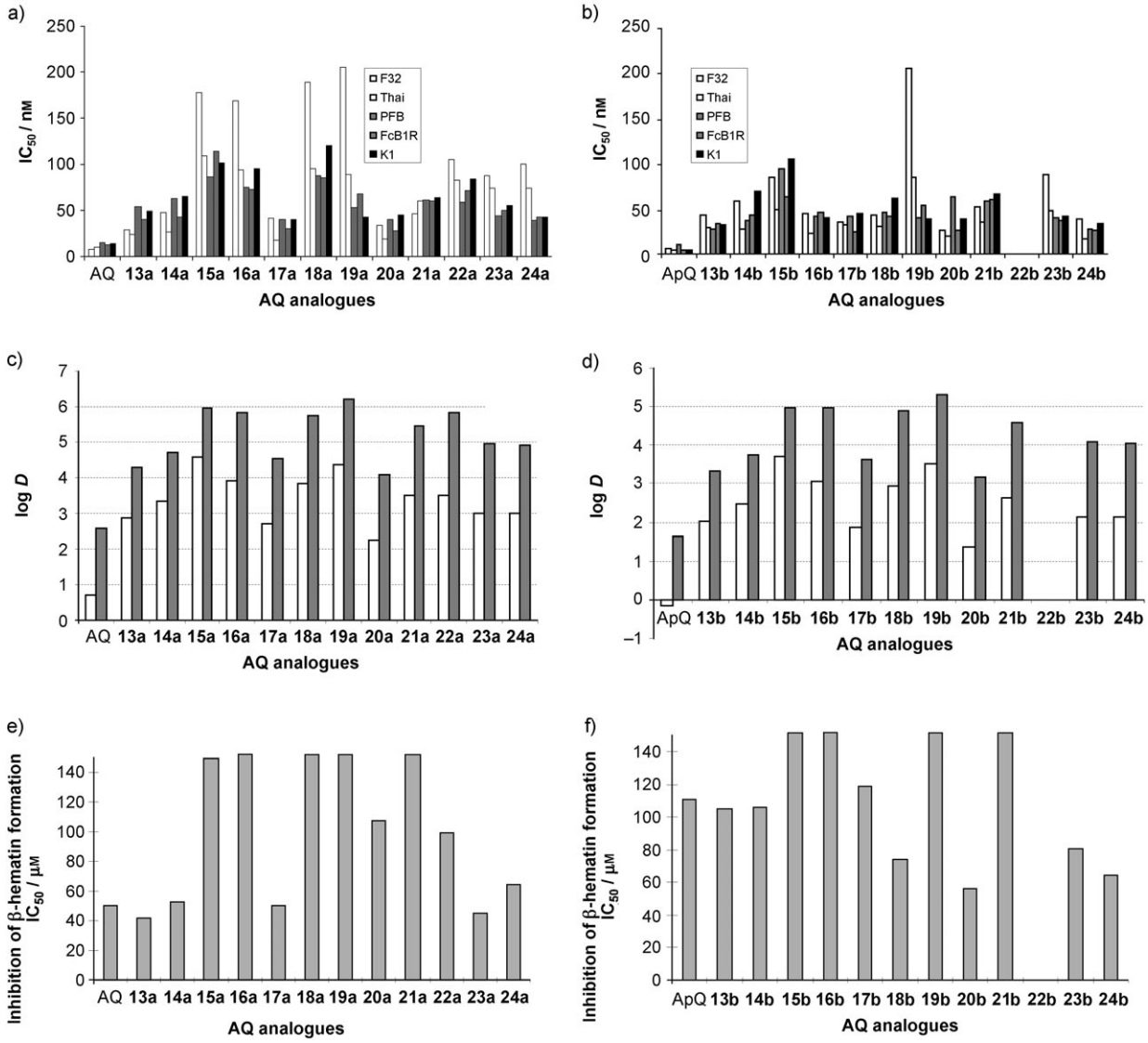


Figure 6. Comparison between in vitro antimalarial activity, lipophilicity, and in vitro inhibition of β-hematin formation of aromatic compounds. a), b): Efficiency (IC_{50}) of a) AQ and b) ApQ analogues to inhibit the growth of parasites expressing varying degrees of resistance to CQ. c), d): Calculated log *D* at pH 5.0 (white bars) and pH 7.4 (gray bars) of c) AQ and d) ApQ analogues. e),f): In vitro inhibition of β-hematin formation of e) AQ and f) ApQ analogues.

for ApQ analogues bearing *p*-trifluoromethoxy (**18b**), *p*-methoxy (**17b**), *p*-acetyl (**20b**), or *p*-fluoro (**24b**) groups regardless of the strain tested, and *m*-fluoro (**23b**) and *p*-benzyloxy (**19b**) groups toward the K1 strain. Replacement of the *p*-fluoro substituent with a *p*-chloro group led to less active compounds (compare **21** and **24**). Halogenated analogues of both series had intermediate activity, with IC_{50} values in the range of 50–100 nM.

The introduction of a more bulky or hydrophobic substituent such as *p*-*tert*-butyl, *p*-trifluoromethyl, or *p*-trifluoromethoxy groups in the AQ analogues led to a dramatic decrease in antiplasmodial activity. In both series, except for aromatic compounds substituted with very hydrophobic substituents or the thienyl analogue **25a**, IC_{90} values remained below 100 nM against the K1 strain (Table 1).

The cytotoxicity values (CC_{50}) determined for these compounds allowed us to calculate the selectivity index (the ratio of cytotoxicity and antimalarial activity determined with the CQ-resistant strain K1). Generally, ApQ analogues appeared more cytotoxic than their AQ counterparts (Table 1). Alkyl ApQ analogues presented similar CC_{50} values to that of the parent ApQ, whereas alkyl analogues of AQ were more toxic than AQ itself, except in the case of *n*-butyl compound **12a**, which presented surprisingly low cytotoxicity.

Regardless of their substitution, aryl ApQ analogues showed high cytotoxicity, with CC_{50} values around 2–10 μM. This was not systematically observed for the aryl-AQ analogues. In this family, halogenated compounds substituted with *p*-chloro, *p*-trifluoromethyl, *p*-trifluoromethoxy, or even *p*-acetyl derivatives presented low cytotoxicity (CC_{50} ~50–85 μM), whereas more

Table 1. In vitro antimalarial activity against CQ-sensitive and CQ-resistant K1 strains,[a] cytotoxicity toward MRC-5 cells, and selectivity index.

Compound	R	F32		K1		Cytotoxicity	SI (K1)[d]
		$IC_{50} \pm SD$[b] [nM]	$IC_{90} \pm SD$[b] [nM]	$IC_{50} \pm SD$[b] [nM]	$IC_{90} \pm SD$[b] [nM]	CC_{50}[c] $\pm SD$[b] [μM]	CC_{50}/IC_{50}
AQ analogues ($NR^1R^2 = NEt_2$):							
AQ	OH	8.1 ± 0.7	28.3 ± 1.3	9.0 ± 0.6	11.8 ± 2.5	29.4 ± 2.1	3267
deOH-AQ	H	19.2 ± 2.0	29.8 ± 0.6	18.1 ± 1.0	31.5 ± 0.8	31.3 ± 0.8	1729
10a[e]	Me	18.5 ± 4.6	27.0 ± 1.9	16.1 ± 1.0	30.7 ± 5.3	14.2 ± 0.7	1292
11a[e]	Et	18.0 ± 1.9	30.5 ± 1.7	25.2 ± 1.8	43.0 ± 4.7	18.1 ± 1.2	718
12a	*n*Bu	38.5 ± 3.1	104.1 ± 26.3	64.5 ± 3.6	97.8 ± 2.8	91.0 ± 9.1	1411
13a[e]	C_6H_5	28.5 ± 7.9	45.6 ± 11.6	48.8 ± 7.6	81.6 ± 7.6	13.7 ± 5.2	281
14a[e]	pCH_3-C_6H_4	46.9 ± 3.3	74.3 ± 3.5	65.1 ± 6.8	108 ± 19.5	10.4 ± 0.1	160
15a[e]	p(*t*Bu)-C_6H_4	177.7 ± 5.8	318.1 ± 5.0	101.8 ± 9.0	157.9 ± 11.6	4.2 ± 0.4	41
16a[e]	pCF_3-C_6H_4	168.3 ± 5.4	319.8 ± 11.2	95.1 ± 11.5	146.8 ± 13.3	86.7 ± 12.6	912
17a[e]	pCH_3O-C_6H_4	40.9 ± 2.5	63.5 ± 1.3	40.3 ± 3.8	70.9 ± 9.9	10.3 ± 0.2	256
18a	pCF_3O-C_6H_4	188.4 ± 9.2	345.4 ± 12.5	120.0 ± 29.5	194.9 ± 52.9	62.0 ± 1.3	517
19a	*p*BnO-C_6H_4	205.1 ± 10.6	366.6 ± 6.6	43.1 ± 11.5	70.3 ± 11.9	3.5 ± 1.1	81
20a[e]	pCH_3CO-C_6H_4	34.0 ± 11.1	54 ± 16.8	45.5 ± 4.5	78.4 ± 9	51.3 ± 1.7	1128
21a[e]	*p*Cl-C_6H_4	45.8 ± 4.1	75.3 ± 4	63.2 ± 1.4	102.7 ± 2.3	54.6 ± 8.3	864
22a[e]	*o*F-C_6H_4	104.9 ± 22.0	160.0 ± 44.4	84.2 ± 6.7	129.0 ± 9.7	5.8 ± 0.7	69
23a	*m*F-C_6H_4	87.3 ± 2.2	134.6 ± 1.6	54.9 ± 17.8	82.9 ± 25.5	28.2 ± 2.5	513
24a[e]	*p*F-C_6H_4	99.7 ± 14.9	167.5 ± 43.2	42.7 ± 4.5	65.5 ± 4.4	17.4 ± 0.2	408
25a[e]	2-thiophene	38.4 ± 4.3	86.1 ± 23.5	62.4 ± 4.9	140.8 ± 18.8	16.1 ± 0.3	257
26a[e]	2-furan	29.2 ± 13.0	65 ± 7.7	47.5 ± 3.6	90.4 ± 1.6	18.6 ± 0.8	392
ApQ analogues (NR^1R^2 = pyrrolidine):							
ApQ	OH	5.7 ± 1.5	10.0 ± 2.4	4.2 ± 1.4	9.6 ± 3.6	23.5 ± 1.3	5595
deOH-ApQ	H	11.8 ± 3.5	15.5 ± 0.9	8.7 ± 2.4	22.5 ± 6.4	29.8 ± 6.2	3425
10b[e]	Me	10.0 ± 0.6	48.0 ± 11.7	10.3 ± 4.4	18.2 ± 0.6	21.3 ± 3.4	1379
11b[e]	Et	16.6 ± 6.6	21.8 ± 5.2	11.8 ± 0.4	19.2 ± 1.1	18.9 ± 3.8	1602
12b	*n*Bu	32.9 ± 3.5	42.5 ± 11.3	20.4 ± 4.8	44.6 ± 12.8	14.5 ± 2.7	711
13b[e]	C_6H_5	43.2 ± 3.8	69.7 ± 30.7	32.6 ± 8.2	58.4 ± 3.6	7.5 ± 0.3	230
14b[e]	pCH_3-C_6H_4	59.3 ± 11.8	112 ± 4.4	70.0 ± 2.3	118.2 ± 2.7	8.1 ± 0.2	116
15b[e]	p(*t*Bu)-C_6H_4	84.9 ± 3.8	134.3 ± 5.8	104.8 ± 29.8	203 ± 35.8	5.2 ± 0.1	50
16b[e]	pCF_3-C_6H_4	44.6 ± 5.6	86.3 ± 29.3	40.6 ± 6.3	76.3 ± 5	6.4 ± 0.1	158
17b[e]	pCH_3O-C_6H_4	35.4 ± 1.3	65 ± 1.4	44.2 ± 2.1	79.5 ± 7.9	9.0 ± 0.6	203
18b	pCF_3O-C_6H_4	42.5 ± 2.7	60.6 ± 13.2	62.5 ± 13.4	104.8 ± 19.6	5.8 ± 0.3	93
19b	*p*BnO-C_6H_4	205.7 ± 4.1	343.6 ± 10.4	39.1 ± 4.7	62.4 ± 5.0	2.2 ± 0.1	55
20b[e]	pCH_3CO-C_6H_4	25.6 ± 9.7	44.1 ± 15.7	38.1 ± 4.4	59.5 ± 9.3	10.8 ± 0.4	283
21b[e]	*p*Cl-C_6H_4	52.1 ± 4.0	88.7 ± 13.6	66.7 ± 3.2	106.1 ± 2	10.6 ± 0.2	158
22b[e,f]	*o*F-C_6H_4	nd	nd	nd	nd	nd	nd
23b	*m*F-C_6H_4	87.7 ± 17.3	209.6 ± 5.5	41.0 ± 2.2	60.4 ± 2.2	8.3 ± 0.2	202
24b[e]	*p*F-C_6H_4	39.3 ± 2.5	57.8 ± 15.8	33.8 ± 1.7	59.3 ± 1.3	8.7 ± 0.4	257
25b[e]	2-thiophene	57.4 ± 12.0	64.2 ± 4.2	83.7 ± 3.6	96.8 ± 3.5	13.1 ± 4.5	157
26b[e]	2-furan	38.6 ± 7.7	44.5 ± 18.7	54.4 ± 5.2	81.3 ± 4.7	21.8 ± 1.1	401

[a] Parasites were considered resistant to CQ for $IC_{50} > 100$ nM. [b] Number of experiments: between 3 and 6. [c] CC_{50} is the IC_{50} value for cytotoxicity calculated on the basis of three experiments. [d] Selectivity index, calculated as the ratio between CC_{50} and IC_{50} values toward the K1 strain of *P. falciparum*. [e] See reference [23]. [f] Not determined.

hydrophobic and bulky substituents provided highly toxic compounds (**15a**, **19a**). Indeed, if all these derivatives presented selectivity index values inferior to those of the reference compounds AQ and ApQ, interesting values (>1000) were determined in the case of analogues **10a**, **12a**, **10b**, and **11b**.

AQ and ApQ showed high differences in their ability to inhibit β-hematin formation in vitro, with ApQ being much less efficient. ApQ analogues **10b**–**26b** were also found to be less efficient inhibitors than AQ analogues **10a**–**26a**. Relatively small changes at the Mannich amino side chain (pyrrolidino or diethylamino groups) are correlated with a significant modulation in the compounds' ability to inhibit β-hematin formation in vitro, and thus in their interaction with the tetrapyrrolic porphyrin aromatic system.

Concerning the ability of AQ analogues to inhibit β-hematin formation, compounds can be grouped into four classes: 1) Good inhibitors, with IC_{50} values around 40–50 μM (close to those determined for AQ); they are derivatives substituted with C_6H_5 (**13a**), pCH_3-C_6H_4 (**14a**), pCH_3O-C_6H_4 (**17a**), mF-C_6H_4 (**23a**), pF-C_6H_4 (**24a**), and 2-thiophene (**24a**)—compounds generally bearing less bulky substituents. 2) Compounds with inhibitory activity close to that of CQ (IC_{50} values between 70 and 81 μM); these include dehydroxy-AQ (deOH-AQ) and the 2-furan-substituted analogue **26a**. 3) Analogues that are slightly less active than CQ, with IC_{50} values between 90 and 107 μM, such as the alkyl compounds **10a** and **11a**, and derivatives substituted with oF-C_6H_4 (**22a**) and pCH_3CO-C_6H_4 (**20a**). 4) Weak inhibitors ($IC_{50}>150$ μM) such as the analogues substituted with p(tBu)-C_6H_4 (**15a**), pCF_3-C_6H_4 (**16a**), pCF_3O-C_6H_4 (**18a**), pBnO-C_6H_4 (**19a**), and pCl-C_6H_4 (**21a**); their weak inhibitory activity is probably due to the relatively large size of substituents.

ApQ analogues can also be classified according to their capacity to inhibit β-hematin formation: 1) Products that are better inhibitors than CQ, such as compounds substituted with pCH_3CO-C_6H_4 (**20b**) and pF-C_6H_4 (**24b**) (IC_{50}: 56 and 65 μM, respectively). 2) Products with activity similar to that of CQ (IC_{50} range: 74–85 μM), such as the ApQ alkyl derivatives **10b** and **11b**, and the aryl derivatives pCF_3O-C_6H_4 (**18b**), mF-C_6H_4 (**23b**), 2-thiophene (**25b**), and 2-furan (**26b**). 3) ApQ, deOH-AQ, and the derivatives substituted with C_6H_5 (**13b**), pCH_3-C_6H_4 (**14b**), and pCH_3O-C_6H_4 (**17b**) present similar but slightly lower activity than CQ. 4) Products with poor inhibitory activity ($IC_{50}>$ 150 μM), substituted with p(tBu)-C_6H_4 (**15b**), pCF_3-C_6H_4 (**16b**), pBnO-C_6H_4 (**19b**), and pCl-C_6H_4 (**21b**).

Based on selectivity index values and antimalarial activity of all the 4′-aryl- and alkyl-substituted analogues, compound **10b** was selected for further in vivo tests. Its activity was compared with that of the parent compound AQ in mice infected with *P. berghei* by i.p. administration (50 mg kg^{-1}; Peters test). Untreated control animals generally die between 8 and 12 days following infection. Drug activity was evaluated by the decrease in parasitemia at day 4 and day 11. Compound **10b** presented good in vivo antimalarial activity, very close to that of the reference compound AQ, with >99% decrease in parasitemia observed at day 4 and day 11. No parasites were detected in the AQ-treated mice at day 4 and day 11.

Discussion

Relative to other antimalarial agents, resistance to the 4-aminoquinolines developed and spread relatively slowly, because resistance would imply complex point mutations of the genes that encode transport proteins. Amodiaquine maintains its efficiency in many endemic zones and is still recommended by the WHO, but numerous studies have highlighted its potential toxicity in prophylactic use. Nevertheless, relatively small structural modifications of AQ-like structures can lead to a significant increase in antimalarial activity against CQ-resistant strains and can impart greater metabolic stability to these compounds.

The 4-aminoquinoline drug CQ accumulates in the acidic food vacuole of the intraerythrocytic malaria parasite, and prevents the detoxification of hematin released during hemoglobin digestion. The mechanism of interaction between 4-aminoquinoline drugs and β-hematin has been widely investigated.[25,26] In all the proposed models, differences have been illustrated between CQ and AQ, with the introduction of the aromatic cycle increasing AQ lipophilicity and side chain rigidity, and favoring π–π stacking interactions.[4e,13,25b]

The metabolism of AQ has been studied.[20b,27,28] Its protein conjugates are involved in both toxicity and clearance,[28] and their formation has been so far prevented by fluorine substitution at the 4′-position of the aromatic nucleus[21c] or by introduction of an alkyl or aryl substituent at the 5′-position.[17,18]

In this study, our strategy consisted of replacing the 4′-hydroxy group with various hydrophobic alkyl, aryl, or heteroaryl substituents. Thanks to a previously described study of the cross-coupling Suzuki–Miyaura reaction in this series, we were able to synthesize alkyl, aryl, or heteroaryl analogues according to a similar synthetic approach.[23]

This kind of structural modification might a priori increase compound lipophilicity, π–π stacking interactions, and prevent metabolic oxidation by blocking the 4′-position. Moreover, as 4′-dehydroxy analogues maintain antimalarial activity, we wanted to know if this activity is due to re-oxidation of deOH-AQ into AQ or an intrinsic activity. As resistance to AQ has been described to involve mutations of the *PfCRT* gene,[11,13] the compounds were evaluated with strains that present various CQ-resistance properties.

The observed differences in the activity profiles between the AQ analogues and their corresponding ApQ counterparts confirm that relatively small structural changes in the amino side chain determine significant modifications in antimalarial activity and the ability to inhibit β-hematin formation in vitro (Figure 6e and 6f).

Alkyl-AQ and ApQ analogues substituted with short chains, such as methyl (**10a,b**) or ethyl groups (**11a,b**), show high antimalarial activity against both CQ-sensitive and CQ-resistant *P. falciparum* strains, with IC_{50} values close to those of the dehydroxy analogues. This activity could be explained by similar interactions with heme, leading to a similarly high inhibition of β-hematin formation and similar accumulation into the food vacuole (calculated data not shown). By using ACDLabs software, we were able to calculate pK_a values of our compounds

and thus estimate their ability to accumulate into the acidic food vacuole of the parasite.[29] Interestingly, the 4′-methyl- and 4′-ethyl-substituted analogues are also associated with relatively low cytotoxicity and high selectivity index values (>1000). The high antimalarial activity of these alkyl analogues was similar to the results obtained for 5′-alkyl derivatives of AQ described by Raynes et al.[17a]

In comparison with their counterparts bearing shorter alkyl chains, *n*-butyl-substituted derivatives **12a** and **12b** present a decreased in activity, especially against the K1 CQ-resistant strain; this can be correlated with an increase in lipophilicity (see log *D* values, Figure 6c and 6d).

All the 4′-aryl- or heteroaryl-AQ analogues tested were less active than the 4′-methyl- and 4′-ethyl-AQ derivatives. This seems to be correlated with a potential decrease in the ability to accumulate into the food vacuole (calculated data not shown) and to inhibit β-hematin formation. In general, ApQ analogues are more active than their AQ counterparts, together with a decreased ability to inhibit the formation of β-hematin.

In this series of aromatic compounds, the classical mechanism of action of 4-aminoquinolines seems to be generally adopted: compounds that inhibit the formation of β-hematin more efficiently are better inhibitors of parasite growth. This is the case for phenyl compounds **13a,b**, *p*-methoxy derivatives **17a,b**, and fluoro compounds **23a,b** and **24a,b**.

The case of fluoro compounds is exemplary. Even if one considers a priori that the fluorine atom acts mostly by its electron-withdrawing properties, and not by inducing significant steric effects, a certain influence through steric hindrance cannot be ruled out, especially regarding the antimalarial activity against the F32 strain. The presence of the fluorine atom in the *ortho* position (compound **22a**) increases the torsion angle of the biphenyl unit (as clearly illustrated by comparison of the solid-state structures determined in the case of compounds **22a** and **20b**; see information for supplementary crystallographic data in the Experimental Section below). This leads to a decrease in the capacity to interact with heme, and to inhibit β-hematin formation and thus antimalarial activity.

Regarding the compounds' ability to act as inhibitors of β-hematin formation in vitro, small and polar substituents seem to be more favorable, as is the case for analogues **13a**, **14a**, **17a**, **23a**, **24a**, and **25a**, bearing the respective substituents: C_6H_5-, *p*CH_3-C_6H_4-, *p*CH_3O-C_6H_4-, *m*F-C_6H_4-, *p*F-C_6H_4-, and 2-thiophene. This is probably due to interactions with the carboxylate moiety of heme.

On the other hand, weak inhibitors of β-hematin formation, such as analogues **15a**, **16a**, **18a**, and **19a** are less efficient antimalarial compounds, particularly toward the F32 CQ-sensitive strain. These compounds are more lipophilic and bear bulkier substituents, which disfavor interaction with heme. The more lipophilic thiophene analogues also belong to this case. The decrease in activity of these hydrophobic analogues seems contradictory with our previous results.[22a]

Interestingly, chloro-substituted aryl analogues **21a** and **21b** provide an exception to this general trend. Indeed, while they are thought to accumulate into the food vacuole less than aryl compounds **13a,b** or **14a,b** and display almost the same antimalarial activity, their inhibition of β-hematin formation is threefold less potent ($IC_{50} > 150$ μM). These results suggest that additional mechanisms may be involved for these compounds. Moreover, these compounds have IC_{50} values against the K1 strain that are sevenfold higher than that of AQ, which is not consistent with other studies[4a] in which TBQ or 5′-(*p*-chlorophenyl)-AQ were found to be as active as AQ against the K1 strain.

Generally, the 4′-aryl- and 4′-heteroaryl-ApQ analogues seem to be more cytotoxic than their AQ counterparts. Therefore, even if, in some cases, interesting antimalarial activities were determined, the measured CC_{50} values do not encourage further development of ApQ derivatives.

Conclusions

An efficient and convergent synthetic strategy that involves a Suzuki–Miyaura cross-coupling reaction has been developed for the synthesis of these 4′-alkyl, aryl, and heteroaryl derivatives of amodiaquine. Only six steps were necessary to obtain the target compounds.

A detailed SAR study was carried out regarding the influence of the substituent at the 4′-position of the amino side chain. The role of the amino side chain was of primary importance for the inhibition of β-hematin formation. No direct correlation could be established between antimalarial activity and the compounds' ability to inhibit β-hematin formation or the calculated vacuolar accumulation ratios.

The aliphatic derivatives are more active than the aromatic derivatives. The IC_{50}, IC_{90}, and SI values indicate compounds **10a**, **10b**, and **11b** as promising candidates for further development. Compound **10b** has shown in vivo antimalarial activity, although inferior to parent compound AQ. These new promising antimalarial compounds might be considered for further investigation as potentially safer alternatives to amodiaquine. The introduction of further functionalized small and polar alkyl substituents at the 4′-position is currently in progress.

Experimental Section

Chemistry

All reactions were monitored by thin-layer chromatography (TLC) carried out on E. Merck silica gel plates (60 F_{254}, 0.2 mm), with UV light for visualization. Preparative TLC was performed on 20× 20 cm plates prepared in the laboratory covered with silica gel from Merck (60 F_{254}), visualized with UV light; the compounds were eluted with CH_2Cl_2/MeOH/NH_4OH (75:25:4). All melting points were determined on a Büchi melting point apparatus, and are uncorrected. ^1H and ^{13}C NMR spectra were obtained with a Bruker 300 MHz spectrometer; chemical shifts (δ) were expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. Carbon atom assignments were deduced after 2D experiments were performed. Mass spectra were recorded on a MALDI-TOF Voyager-DE-STR spectrometer. Final compound purity was verified by using two types of HPLC column: C18 Deltapak ($C_{18}N$) and C4 Interchrom UP5WC4-25QS (C_4). Analytical HPLC was performed on a Shimadzu system

equipped with a UV detector set at $\lambda = 254$ nm. Compounds were dissolved in buffer B or MeOH and injected through a 50-μL loop. The following eluent systems were used: buffer A (H_2O/TFA 100:0.05) and buffer B (CH_3CN/H_2O/TFA 80:20:0.05). HPLC retention times (t_R) were obtained at flow rates of 1 mL min^{-1}, under the following conditions: for the 10-min method, a gradient run from 100% buffer A for 30 s, then to 100% buffer B for the next 8 min; for the 40-min method: a gradient run from 100% buffer A for 1 min, then to 100% buffer B for the next 30 min. Reagents were obtained from Acros, Aldrich, Lancaster, Novabiochem, and Avocado. Abbreviation for quinoline: Quin.

(2-Butyl-5-nitrobenzyl)diethylamine 2a. A suspension of bromide **1a**[23] (200 mg, 0.697 mmol), *n*-butylboronic acid (127 mg, 2 eq), $Pd(OAc)_2$ (24 mg, 0.15 eq), P(*o*-tol)$_3$ (63 mg, 0.3 eq), and K_2CO_3 (289 mg, 3 eq) in a mixture of THF (5 mL) and H_2O (0.5 mL) was heated at 65 °C under an inert atmosphere (N_2) for 13 days. The medium was evaporated, solubilized with a saturated aqueous solution of $NaHCO_3$ (50 mL), and extracted in CH_2Cl_2 (5×50 mL). Combined organic layers were dried over $MgSO_4$, filtered on Celite and concentrated. The residue was purified by TLC (*c*Hex/EtOAc/NH_4OH/ 9:1:0.2) then by preparative HPLC (eluents A/B) to yield the expected compound **2a** as a white solid (trifluoroacetate, 37 mg, 20% yield); $R_f = 0.6$ (EtOAc/*c*Hex/NH_4OH 1:9:0.2); ^{1}H NMR (CD_3OD): $\delta = 8.39$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.19 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.56 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 4.38 (2H, s, CH_2), 3.18 (4H, q, N-CH_2, $^3J = 7.0$ Hz), 2.83 (2H, dd, Ar-CH_2, $^3J = 7.8$ Hz), 1.54–1.62 (2H, m, CH_2), 1.33–1.46 (2H, m, CH_2), 1.31 (6H, t, CH_3, $^3J = 7.0$ Hz), 0.95 ppm (3H, t, CH_3, $^3J = 7.2$ Hz); ^{13}C NMR (CD_3OD): $\delta = 131.4$ (Ar-C_3), 125.8 (Ar-C_6), 124.1 (Ar-C_4), 52.6 (CH_2), 47.2 (2C, CH_2), 33.0 (CH_2), 32.4 (CH_2), 22.3 (CH_2), 13.0 (CH_3), 8.3 ppm (2C, CH_3); MS *m/z*: 265.2 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 99%, $t_R = 4.8$ min.

1-(2-Butyl-5-nitrobenzyl)pyrrolidine 2b. Synthesized from compound **1b**[23] (200 mg, 0.701 mmol), *n*-butylboronic acid (107 mg), $Pd(OAc)_2$ (32 mg), P(*o*-tol)$_3$ (84 mg), and K_2CO_3 (291 mg) according to the same protocol as **2a** (reflux for 10 days). The residue was purified by TLC (pentane/EtOAc/NH_4OH 9.5:0.5:0.2), then by preparative HPLC (eluents A/B) to yield the expected compound **2b** as a white solid (trifluoroacetate, 52 mg, 20% yield); $R_f = 0.6$ (pentane/EtOAc/NH_4OH 9.5:0.5:0.2); ^{1}H NMR (CD_3OD): $\delta = 8.41$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.19 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.56 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 4.54 (2H, s, CH_2), 3.10–3.80 (4H, m, N-CH_2), 2.84 (2H, dd, Ar-CH_2, $^3J = 7.5$ Hz), 1.90–2.30 (4H, m, CH_2), 1.52–1.62 (2H, m, CH_2), 1.34–1.46 (2H, m, CH_2), 0.94 ppm (3H, t, CH_3, $^3J = 7.2$ Hz); ^{13}C NMR (CD_3OD): $\delta = 131.4$ (Ar-C_3), 125.6 (Ar-C_6), 124.3 (Ar-C_4), 54.0 (2C, N-CH_2), 53.8 (CH_2), 33.1 (CH_2), 32.3 (Ar-CH_2), 22.6 (2C, CH_2), 22.3 (CH_2), 13.0 ppm (CH_3); MS *m/z*: 263.2 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 95%, $t_R = 4.6$ min.

Suzuki–Miyaura cross-coupling reaction with aryl boronic acids: general procedure A. An aqueous solution of Na_2CO_3 (2 M, 1 mL) and EtOH (2 mL) were added to a suspension of bromide **1a** or **1b**[23] (1 equiv), boronic acid (2 equiv), $Pd(OAc)_2$ (7.5%), P(*o*-tol)$_3$ (15%), and N(*n*Bu)$_4$Br (20%) in toluene (3 mL) under inert (N_2) atmosphere. The reaction medium was heated at 65 °C, and the reaction progress was monitored by TLC and HPLC. The medium was evaporated, solubilized with an aqueous saturated solution of $NaHCO_3$ (50 mL), and extracted in CH_2Cl_2 (5×50 mL). Combined organic layers were dried over $MgSO_4$, filtered on Celite and concentrated. The residue was purified by TLC.

Diethyl-(4-nitro-4′-trifluoromethoxybiphenyl-2-ylmethyl)amine 3a. White solid (49% yield); $R_f = 0.5$ (*c*Hex/EtOAc/NH_4OH 9:1:0.2); mp: 26 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.56$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.13 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.5$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.36 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.5$ Hz), 7.35 (2H, m, Ph), 7.30 (2H, m, Ph), 3.52 (2H, s, CH_2), 2.46 (4H, q, N-CH_2, $^3J = 7.1$ Hz), 0.92 ppm (6H, t, CH_3, $^3J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 130.8$ (Ar-C_3), 130.5 (2C, Ph), 124.9 (Ar-C_6), 121.6 (Ar-C_4), 120.8 (2C, Ph), 54.6 (CH_2), 46.9 (2C, N-CH_2), 11.7 ppm (2C, CH_3); MS *m/z*: 369.1 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 98%, $t_R = 5.1$ min.

1-(4-Nitro-4′-trifluoromethoxybiphenyl-2-ylmethyl)pyrrolidine 3b. Yellow solid (80% yield); $R_f = 0.4$ (*n*Hex/EtOAc/NH_4OH 9:1:0.2); mp: 73 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.45$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.15 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.45 (2H, m, Ph), 7.39 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 7.30 (2H, m, Ph), 3.56 (2H, s, CH_2), 2.45–2.49 (4H, m, N-CH_2), 1.74–1.79 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): $\delta = 130.8$ (Ar-C_3), 130.6 (2C, Ph), 124.7 (Ar-C_6), 121.6 (Ar-C_4), 120.6 (2C, Ph), 57.0 (CH_2), 53.7 (2C, N-CH_2), 23.5 ppm (2C, CH_2); MS *m/z*: 367.1 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 99%, $t_R = 5.3$ min.

(4′-Benzyloxy-4-nitrobiphenyl-2-ylmethyl)diethylamine 4a. Yellow solid (67% yield); $R_f = 0.5$ (*n*Hex/EtOAc/NH_4OH 8:2:0.2); mp: 55 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.57$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.08 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.34–7.49 (5H, m, Ph), 7.33 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 7.24 (2H, m, Ph), 7.06 (2H, m, Ph), 5.13 (2H, s, O-CH_2), 3.53 (2H, s, 7-CH_2), 2.45 (4H, q, N-CH_2, $^3J = 7.1$ Hz), 0.94 ppm (6H, t, CH_3, $^3J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 130.8$ (Ar-C_3), 130.3 (2C, Ph), 128.7 (2C, Ph), 128.1 (Ph), 127.6 (2C, Ph), 124.7 (Ar-C_6), 121.3 (Ar-C_4), 114.6 (2C, Ph), 70.1 (O-CH_2), 54.5 (CH_2), 47.0 (2C, N-CH_2), 11.9 ppm (2C, CH_3); MS *m/z*: 391.1 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 98%, $t_R = 5.8$ min.

1-(4′-Benzyloxy-4-nitrobiphenyl-2-ylmethyl)pyrrolidine 4b. Yellow solid (65% yield); $R_f = 0.4$ (*n*Hex/EtOAc/NH_4OH 8:2:0.2); mp: 84 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.45$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.11 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.32–7.49 (8H, m, Ar-H$_3$, Ph), 7.05 (2H, m, Ph), 5.13 (2H, s, O-CH_2), 3.60 (2H, s, CH_2), 2.45–2.50 (4H, m, N-CH_2), 1.70–1.83 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): $\delta = 130.7$ (Ar-C_3), 130.3 (2C, Ph), 128.5 (2C, Ph), 128.0 (Ph), 127.4 (2C, Ph), 124.6 (Ar-C_6), 121.4 (Ar-C_4), 114.5 (2C, Ph), 70.0 (Ph), 57.0 (CH_2), 53.7 (2C, N-CH_2), 23.5 ppm (2C, CH_2); MS *m/z*: 389.3 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 97%, $t_R = 5.8$ min.

Diethyl-(3′-fluoro-4-nitrobiphenyl-2-ylmethyl)amine 5a. White solid (43% yield); $R_f = 0.5$ (*c*Hex/EtOAc/NH_4OH 9:1:0.2); mp: 70 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.60$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.13 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.39–7.46 (1H, m, Ph), 7.36 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 7.05–7.16 (3H, m, Ph), 3.55 (2H, s, CH_2), 2.48 (4H, q, N-CH_2, $^3J = 7.1$ Hz), 0.95 ppm (6H, t, CH_3, $^3J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 130.6$ (Ar-C_3), 129.9 (d, Ph, $^4J_{CH,F} = 8.3$ Hz), 124.8 (Ar-C_6), 124.6 (Ph), 121.5 (Ar-C_4), 115.6 (d, Ph, $^3J_{CH,F} = 21.4$ Hz), 114.9 (d, Ph, $^3J_{CH,F} = 20.7$ Hz), 54.3 (CH_2), 46.7 (2C, N-CH_2), 11.5 ppm (2C, CH_3); MS *m/z*: 302.2 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 99%, $t_R = 4.4$ min.

1-(3′-Fluoro-4-nitrobiphenyl-2-ylmethyl)pyrrolidine 5b. White solid (58% yield); $R_f = 0.6$ (*n*Hex/EtOAc/NH_4OH 8:2:0.2); mp: 82 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.45$ (1H, d, Ar-H$_6$, $^4J_{6,4} = 2.4$ Hz), 8.14 (1H, dd, Ar-H$_4$, $^3J_{4,3} = 8.4$ Hz, $^4J_{4,6} = 2.4$ Hz), 7.40–7.46 (1H, m, Ph), 7.39 (1H, d, Ar-H$_3$, $^3J_{3,4} = 8.4$ Hz), 7.14–7.22 (3H, m, Ph), 3.57 (2H, s, CH_2), 2.45–2.50 (4H, m, N-CH_2), 1.49–1.79 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): $\delta = 130.8$ (Ar-C_3), 129.9 (d, Ph, $^4J_{CH,F} = 8.3$ Hz), 124.9 (Ph), 124.8 (Ar-C_6), 121.7 (Ar-C_4), 116.4 (d, Ph, $^3J_{CH,F} = 22.3$ Hz), 115.0 (d, Ph, $^3J_{CH,F} = 21.0$ Hz), 57.1 (CH_2), 53.9 (2C, N-CH_2), 23.7 ppm (2C, CH_2); MS *m/z*: 301.2 $[M+H]^+$; HPLC (C_{18}, 10 min), HPLC$_{prep}$: 99%, $t_R = 4.5$ min.

Reduction of the nitro group: general procedure B. A solution of $SnCl_2$ (4 equiv) in THF with HCl (1 M, 3 equiv) was added to a solution of nitro compound (1 equiv) in THF. After stirring at reflux, the mixture was concentrated, the residue was made alkaline with an aqueous solution of $NaHCO_3$ (pH 8), and the aqueous layer was extracted with CH_2Cl_2 (5×50 mL). The organic layers were then combined, dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by TLC.

4-Butyl-3-diethylaminomethylphenylamine 6a. Yellow oil (44% yield); R_f=0.4 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=6.92 (1H, d, Ar-H_3, $^3J_{3,4}$=8.0 Hz), 6.81 (1H, d, Ar-H_6, $^4J_{6,4}$=2.6 Hz), 6.52 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.0 Hz, $^4J_{4,6}$=2.6 Hz), 3.30–3.60 (2H, s broad, NH_2), 3.47 (2H, s, CH_2), 2.49–2.59 (6H, m, Ar-CH_2, 2× 9-CH_2), 1.44–1.53 (2H, m, CH_2), 1.38–1.44 (2H, m, CH_2), 1.04 (6H, t, CH_3, ^3J=7.2 Hz), 0.92 ppm (3H, t, CH_3, ^3J=7.2 Hz); ^{13}C NMR ($CDCl_3$): δ=130.0 (Ar-C_3), 116.5 (Ar-C_6), 113.6 (Ar-C_4), 54.8 (CH_2), 46.8 (2C, N-CH_2), 33.7 (CH_2), 31.5 (Ar-CH_2), 22.7 (CH_2), 14.0 (CH_3), 11.6 ppm (2C, CH_3); MS *m/z*: 235.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 95%, t_R=3.4 min.

4-Butyl-3-pyrrolidin-1-ylmethylphenylamine 6b. Yellow oil (62% yield); R_f=0.5 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=6.93 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 6.79 (1H, d, Ar-H_6, $^4J_{6,4}$=2.5 Hz), 6.53 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.5 Hz), 3.60 (2H, s, CH_2), 3.35–3.55 (2H, broad s, NH_2), 2.55–2.62 (6H, m, Ar-CH_2, N-CH_2), 1.54–1.85 (4H, m, CH_2), 1.42–1.49 (2H, m, CH_2), 1.30–1.40 (2H, m, CH_2), 0.92 ppm (3H, t, CH_3, ^3J=7.5 Hz); ^{13}C NMR ($CDCl_3$): δ=130.1 (Ar-C_3), 116.3 (Ar-C_6), 114.0 (Ar-C_4), 57.0 (CH_2), 54.2 (2C, N-CH_2), 33.8 (CH_2), 31.6 (Ar-CH_2), 23.5 (2C, CH_2), 22.7 ppm (CH_2), 14.0 (CH_3); MS *m/z*: 233.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: >99%, t_R=3.2 min.

2-Diethylaminomethyl-4′-trifluoromethoxybiphenyl-4-ylamine 7a. Yellow oil (65% yield); R_f=0.5 (CH_2Cl_2/NH_4OH 10:0.2); ^{1}H NMR ($CDCl_3$): δ=7.29 (2H, m, Ph), 7.20 (2H, m, Ph), 7.02 (1H, d, Ar-H_6, $^4J_{6,4}$=2.3 Hz), 6.98 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 6.62 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.3 Hz), 3.98 (2H, s broad, NH_2), 3.50 (2H, s, CH_2), 2.49 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.92 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=130.1 (2C, Ph), 129.9 (Ar-C_3), 119.4 (2C, Ph), 115.1 (Ar-C_6), 112.8 (Ar-C_4), 53.4 (CH_2), 45.5 (2C, N-CH_2), 10.1 ppm (2C, CH_3); MS *m/z*: 339.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=4.7 min.

2-Pyrrolidin-1-ylmethyl-4′-trifluoromethoxybiphenyl-4-ylamine 7b. Synthesized from compound **3b** (192 mg, 0.523 mmol) and $SnCl_2$ (397 mg) in HCl (1.57 mL) and THF (25 mL) according to general procedure B (reflux for 3 h). The residue was purified by TLC (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2) to yield compound **7b** as a yellow oil (146 mg, 83% yield); R_f=0.5 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=7.37 (2H, m, Ph), 7.19 (2H, m, Ph), 7.01 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 6.92 (1H, d, Ar-H_6, $^4J_{6,4}$=2.5 Hz), 6.60 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.5 Hz), 3.70–3.90 (2H, s broad, NH_2), 3.47 (2H, s, CH_2), 2.43–2.47 (4H, m, N-CH_2), 1.68–1.79 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=131.0 (2C, Ph), 130.9 (Ar-C_3), 120.2 (2C, Ph), 115.9 (Ar-C_6), 113.5 (Ar-C_4), 57.4 (CH_2), 53.8 (2C, N-CH_2), 23.4 ppm (2C, CH_2); MS *m/z*: 337.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 95%, t_R=4.7 min.

4′-Benzyloxy-2-diethylaminomethylbiphenyl-4-ylamine 8a. Yellow oil (76% yield); R_f=0.5 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=7.25–7.46 (5H, m, Ph), 7.19 (2H, m, Ph), 7.01 (1H, d, Ar-H_6, $^4J_{6,4}$=2.5 Hz), 6.98 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 6.96 (2H, m, Ph), 6.55 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.5 Hz), 5.06 (2H, s, O-CH_2), 3.67 (2H, s broad, NH_2), 3.45 (2H, s, CH_2), 2.43 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.91 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=130.8 (Ar-C_3), 130.7 (2C, Ph), 128.5 (2C, Ph), 127.9 (Ph), 127.4 (2C, Ph), 115.6 (Ar-C_6), 114.1 (2C, Ph), 113.3 (Ar-C_4), 69.9 (O-CH_2), 54.4 (CH_2), 46.6 (2C, N-CH_2), 11.5 ppm (2C, CH_3); MS *m/z*: 361.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=5.0 min.

4′-Benzyloxy-2-pyrrolidin-1-ylmethylbiphenyl-4-ylamine 8b. White solid (64% yield); R_f=0.4 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 135 °C; ^{1}H NMR ($CDCl_3$): δ=7.34–7.48 (5H, m, Ph), 7.25 (2H, m, Ph), 7.03 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 6.98 (2H, m, Ph), 6.95 (1H, d, Ar-H_6, $^4J_{6,4}$=2.5 Hz), 6.61 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.5 Hz), 5.09 (2H, s, O-CH_2), 3.70 (2H, s broad, NH_2), 3.53 (2H, s, CH_2), 2.44–2.49 (4H, m, N-CH_2), 1.67–1.80 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=130.9 (Ar-C_3), 130.7 (2C, Ph), 128.5 (2C, Ph), 127.9 (Ph), 127.5 (2C, Ph), 115.7 (Ar-C_6), 114.1 (2C, Ph), 113.4 (Ar-C_4), 69.9 (O-CH_2), 57.3 (CH_2), 53.9 (2C, N-CH_2), 23.4 ppm (2C, CH_2); MS *m/z*: 359.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 98%, t_R=5.0 min.

2-Diethylaminomethyl-3′-fluorobiphenyl-4-ylamine 9a. Yellow oil (72% yield); R_f=0.5 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=7.24–7.33 (1H, m, Ph), 6.94–7.06 (5H, m, Ar-H_3, Ar-H_6, Ph), 6.60 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.5 Hz), 4.00 (2H, s broad, NH_2), 3.48 (2H, s, CH_2), 2.47 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.92 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=129.9 (Ar-C_3), 128.3 (d, Ph, $^3J_{CH,F}$=8.4 Hz), 124.6 (Ph), 115.8 (d, Ph, $^2J_{CH,F}$=20.9 Hz), 115.0 (Ar-C_6), 112.6 (Ar-C_4), 112.2 (d, Ph, $^2J_{CH,F}$=20.9 Hz), 53.6 (CH_2), 45.7 (2C, N-CH_2), 10.4 ppm (2C, CH_3); MS *m/z*: 273.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=3.7 min.

3′-Fluoro-2-pyrrolidin-1-ylmethylbiphenyl-4-ylamine 9b. Yellow oil (71% yield); R_f=0.4 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); ^{1}H NMR ($CDCl_3$): δ=7.24–7.33 (1H, m, Ph), 7.09–7.16 (2H, m, Ph), 7.03 (1H, d, Ar-H_3, $^3J_{3,4}$=8.2 Hz), 6.94–7.01 (1H, m, Ph), 6.92 (1H, d, Ar-H_6, $^4J_{6,4}$=2.4 Hz), 6.60 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.2 Hz, $^4J_{4,6}$=2.4 Hz), 3.77 (2H, s broad, NH_2), 3.50 (2H, s, CH_2), 2.42–2.49 (4H, m, N-CH_2), 1.68–1.78 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=130.8 (Ar-C_3), 129.1 (d, Ph, $^3J_{CH,F}$=8.4 Hz), 125.5 (1C, Ph), 116.7 (d, Ph, $^2J_{CH,F}$=21.1 Hz), 115.9 (Ar-C_6), 113.5 (Ar-C_4), 113.0 (d, Ph, $^2J_{CH,F}$=20.8 Hz), 57.3 (CH_2), 53.9 (2C, N-CH_2), 23.4 ppm (2C, CH_2); MS *m/z*: 271.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=3.8 min.

Aromatic nucleophilic substitution of the 4-chlorine atom in 4,7-dichloroquinoline: general procedure C. A solution of 4,7-dichloroquinoline (1 equiv) in CH_3CN and HCl (1 M, 1 equiv) was added to a solution of aniline (1 equiv) in CH_3CN. After stirring at reflux, the mixture was concentrated and purified by TLC to yield the target compound.

(4-Butyl-3-diethylaminomethylphenyl)-(7-chloroquinolin-4-yl)amine 12a. White–yellow solid (87% yield); R_f=0.4 (CH_2Cl_2/NH_4OH 10:0.2); mp: 147 °C; ^{1}H NMR ($CDCl_3$): δ=8.50 (1H, d, Quin-H_2, $^3J_{2,3}$=5.4 Hz), 8.00 (1H, d, Quin-H_8, $^4J_{8,6}$=2.4 Hz), 7.88 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.42 (1H, dd, Quin-H_6, $^3J_{6,5}$=9.0 Hz, $^4J_{6,8}$=2.4 Hz), 7.40 (1H, d, Ar-H_6, $^4J_{6,4}$=2.4 Hz), 7.19 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 7.12 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.4 Hz), 6.90 (1H, d, Quin-H_3, $^3J_{3,2}$=5.4 Hz), 6.70–6.90 (1H, s broad, NH), 3.57 (2H, s, CH_2), 2.69 (2H, dd, Ar-CH_2, ^3J=7.8 Hz), 2.55 (4H, q, N-CH_2, ^3J=7.2 Hz), 1.51–1.61 (2H, m, CH_2), 1.35–1.46 (2H, m, CH_2), 1.05 (6H, t, CH_3, ^3J=7.2 Hz), 0.97 ppm (3H, t, CH_3, ^3J=7.2 Hz); ^{13}C NMR ($CDCl_3$): δ=151.7 (Quin-C_2), 130.2 (Ar-C_3), 128.7 (Quin-C_8), 125.8 (Quin-C_6), 124.1 (Ar-C_6), 121.2 (2C, Ar-C_4, Quin-C_5), 101.9 (Quin-C_3), 54.6 (CH_2), 46.9 (2C, N-CH_2), 33.2 (CH_2), 31.7 (Ar-CH_2), 22.7 (CH_2), 16.0 (CH_3), 11.6 ppm (2C, CH_3); MS *m/z*: 396.4–398.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=4.2 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 98%, t_R=13.7 min; HPLC (C_4, 40 min), $HPLC_{prep}$: 98%, t_R=11.5 min.

(4-Butyl-3-pyrrolidin-1-ylmethylphenyl)-(7-chloroquinolin-4-yl)amine 12b. White solid (47% yield); R_f=0.4 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 182 °C; ^{1}H NMR ($CDCl_3$): δ=8.51 (1H, d, Quin-H_2, $^3J_{2,3}$=5.4 Hz), 8.00 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.87 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.43 (1H, dd, Quin-H_6, $^3J_{6,5}$=9.0 Hz, $^4J_{6,8}$=2.1 Hz), 7.32 (1H, d, Ar-H_6, $^4J_{6,4}$=2.2 Hz), 7.20 (1H, d, Ar-H_3, $^3J_{3,4}$=8.1 Hz), 7.14 (1H, dd, Ar-H_4, $^3J_{4,3}$=8.1 Hz, $^4J_{4,6}$=2.2 Hz), 6.89 (1H, d, Quin-H_3, $^3J_{3,2}$=5.4 Hz), 6.75–6.90 (1H, s broad, NH), 3.65 (2H, s, CH_2), 2.69 (2H, dd, Ar-CH_2, ^3J=7.6 Hz), 2.54–2.59 (4H, m, N-CH_2), 1.75–1.84 (4H, m, CH_2), 1.54–1.64 (2H, m, CH_2), 1.45–1.48 (2H, m, CH_2), 0.97 ppm (3H, t, CH_3, ^3J=7.2 Hz); ^{13}C NMR ($CDCl_3$): δ=152.1 (Quin-C_2), 130.5 (Ar-C_3), 129.0 (Quin-C_8), 126.0 (Quin-C_6), 124.2 (Ar-C_6), 121.7 (Ar-C_4), 121.5 (Quin-C_5), 102.2 (Quin-C_3), 57.4 (CH_2), 54.5 (2C, N-CH_2), 33.5 (CH_2), 32.0 (Ar-CH_2), 23.7 (2C, CH_2), 22.9 (CH_2), 14.2 ppm (CH_3); MS *m/z*: 394.2–396.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: >99%, t_R=4.4 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 98%, t_R=13.6 min; HPLC (C_4, 40 min), $HPLC_{prep}$: 99%, t_R=10.6 min.

(7-Chloroquinolin-4-yl)-(2-diethylaminomethyl-4′-trifluoromethoxybiphenyl-4-yl)amine 18a. White solid (86% yield); R_f=0.7 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 198 °C; ^{1}H NMR ($CDCl_3$): δ=8.58 (1H, d, Quin-H_2, $^3J_{2,3}$=5.3 Hz), 8.04 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.92 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.60 (1H, s, Ar-H_6), 7.47 (1H, dd, Quin-H_6, $^3J_{6,5}$=9.0 Hz, $^4J_{6,8}$=2.1 Hz), 7.37 (2H, d, Ph, $^3J_{2',3'}$=8.8 Hz), 7.24–7.29 (4H, m, Ph, Ar-H_3, Ar-H_4), 7.08 (1H, d, Quin-H_3, $^3J_{3,2}$=5.3 Hz), 6.82 (1H, s broad, NH), 3.49 (2H, s, CH_2), 2.45 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.92 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=151.9 (Quin-C_2), 131.1 (Ar-C_3), 130.9 (2C, Ph), 129.0 (Quin-C_8), 126.2 (Quin-C_6), 123.3 (Ar-C_6), 121.3 (Quin-C_5), 120.5 (2C, Ph), 120.2 (Ar-C_4), 102.6 (Quin-C_3), 54.6 (CH_2), 46.8 (2C, N-CH_2), 11.7 ppm (2C, CH_3); MS *m/z*: 500.3–502.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 98%, t_R=4.8 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 99%, t_R=20.1 min; HPLC (C_4, 40 min), $HPLC_{prep}$: 98%, t_R=20.0 min.

(7-Chloroquinolin-4-yl)-(2-pyrrolidin-1-ylmethyl-4′-trifluoromethoxybiphenyl-4-yl)amine 18b. White solid (97% yield); R_f=0.7 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 187 °C; ^{1}H NMR ($CDCl_3$): δ=8.58 (1H, d, Quin-H_2, $^3J_{2,3}$=5.3 Hz), 8.04 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.90 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.43–7.49 (4H, m, Ar-H_6, Quin-H_6, Ph), 7.25–7.28 (4H, m, Ar-H_3, Ar-H_4, Ph), 7.07 (1H, d, Quin-H_3, $^3J_{3,2}$=5.3 Hz), 6.80 (1H, s broad, NH), 3.54 (2H, s, CH_2), 2.44–2.48 (4H, m, N-CH_2), 1.72–1.76 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=151.9 (Quin-C_2), 131.1 (Ar-C_3), 130.9 (2C, Ph), 129.0 (Quin-C_8), 126.2 (Ar-C_6), 123.4 (Quin-C_6), 121.2 (Quin-C_5), 120.5 (3C, Ph, Ar-C_4), 102.7 (Quin-C_3), 57.4 (CH_2), 54.0 (2C, N-CH_2), 23.5 ppm (2C, CH_2); MS *m/z*: 498.2–500.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=4.8 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 99%, t_R=20.1 min; HPLC (C_4, 40 min), $HPLC_{prep}$: >99%, t_R=19.9 min.

(4′-Benzyloxy-2-diethylaminomethylbiphenyl-4-yl)-(7-chloroquinolin-4-yl)amine 19a. White solid (95% yield); R_f=0.8 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 170 °C; ^{1}H NMR ($CDCl_3$): δ=8.56 (1H, d, Quin-H_2, $^3J_{2,3}$=5.3 Hz), 8.03 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.91 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.62 (1H, d, Ar-H_6, $^4J_{6,4}$=1.5 Hz), 7.35–7.50 (6H, m, Quin-H_6, Ph), 7.20–7.28 (4H, m, Ph, Ar-H_3, Ar-H_4), 7.03–7.07 (3H, m, Quin-H_3, Ph), 6.75–6.90 (1H, s broad, NH), 5.13 (2H, s, O-CH_2), 3.53 (2H, s, CH_2), 2.46 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.94 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=152.0 (Quin-C_2), 131.1 (1C, C-3), 130.6 (2C, Ph), 129.0 (Quin-C_8), 128.6 (2C, Ph), 128.1 (Ph), 127.6 (2C, Ph), 126.0 (Quin-C_6), 123.3 (1C, C-6), 121.3 (Quin-C_5), 120.3 (1C, C-4), 114.4 (2C, Ph), 102.4 (Quin-C_3), 70.1 (O-CH_2), 54.4 (CH_2), 46.9 (2C, N-CH_2), 11.7 ppm (2C, CH_3); MS *m/z*: 522.3–524.3 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=5.1 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 99%, t_R=21.8 min; HPLC (C_4, 40 min), $HPLC_{prep}$: >99%, t_R=21.1 min.

(4′-Benzyloxy-2-pyrrolidin-1-ylmethylbiphenyl-4-yl)-(7-chloroquinolin-4-yl)amine 19b. White solid (93% yield); R_f=0.6 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 154 °C; ^{1}H NMR ($CDCl_3$): δ=8.56 (1H, d, Quin-H_2, $^3J_{2,3}$=5.3 Hz), 8.03 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.88 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.22–7.50 (11H, m, Quin-H_6, Ar-H_3, Ar-H_4, Ar-H_6, Ph), 7.03–7.07 (3H, m, Ph, Quin-H_3), 6.75 (1H, s broad, NH), 5.13 (2H, s, O-CH_2), 3.85 (2H, s, CH_2), 2.44–2.47 (4H, m, N-CH_2), 1.72–1.79 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=151.9 (Quin-C_2), 131.1 (Ar-C_3), 130.6 (2C, Ph), 128.9 (Quin-C_8), 128.5 (2C, Ph), 128.0 (Ph), 127.5 (2C, Ph), 126.0 (Ar-C_6), 123.3 (Quin-C_6), 121.1 (Quin-C_5), 114.3 (2C, Ph), 120.5 (Ar-C_4), 114.3 (2C, Ph), 102.4 (Quin-C_3), 70.1 (O-CH_2), 57.3 (CH_2), 54.0 (2C, N-CH_2), 23.5 ppm (2C, CH_2); MS *m/z*: 520.2–522.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 99%, t_R=5.1 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 98%, t_R=21.9 min; HPLC (C_4, 40 min), $HPLC_{prep}$: >99%, t_R=21.0 min.

7-Chloroquinolin-4-yl)-(2-diethylaminomethyl-3′-fluorobiphenyl-4-yl)amine 23a. White solid (93% yield); R_f=0.7 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 182 °C; ^{1}H NMR ($CDCl_3$): δ=8.57 (1H, d, Quin-H_2, $^3J_{2,3}$=5.4 Hz), 8.04 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.92 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.61 (1H, d, Ar-H_6), 7.48 (1H, d, Quin-H_6, $^3J_{6,5}$=9.0 Hz, $^4J_{6,8}$=2.1 Hz), 7.35–7.42 (1H, m, Ph), 7.24–7.26 (2H, m, Ar-H_3, Ar-H_4), 7.03–7.13 (4H, m, Quin-H_3, Ph), 6.85 (1H, s broad, NH), 3.49 (2H, s, CH_2), 2.45 (4H, q, N-CH_2, ^3J=7.1 Hz), 0.93 ppm (6H, t, CH_3, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$): δ=152.0 (Quin-C_2), 130.9 (Ar-C_3), 129.5 (1C, d, Ph, $^3J_{CH,F}$=8.2 Hz), 129.0 (Quin-C_8), 126.2 (Quin-C_6), 125.3 (Ph), 123.3 (Ar-C_6), 121.3 (Quin-C_5), 120.2 (Ar-C_4), 116.6 (d, Ph, $^2J_{CH,F}$=21.2 Hz), 113.9 (d, Ph, $^2J_{CH,F}$=20.5 Hz), 102.7 (Quin-C_3), 54.6 (CH_2), 46.9 (2C, N-CH_2), 11.8 ppm (2C, CH_3); MS *m/z*: 434.2–436.2 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 98%, t_R=4.1 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: >99%, t_R=18.0 min; HPLC (C_4, 40 min), $HPLC_{prep}$: 99%, t_R=17.5 min.

(7-Chloroquinolin-4-yl)-(3′-fluoro-2-pyrrolidin-1-ylmethylbiphenyl-4-yl)amine 23b. White solid (96% yield); R_f=0.6 (CH_2Cl_2/MeOH/NH_4OH 9.5:0.5:0.2); mp: 182 °C; ^{1}H NMR ($CDCl_3$): δ=8.58 (1H, d, Quin-H_2, $^3J_{2,3}$=5.3 Hz), 8.04 (1H, d, Quin-H_8, $^4J_{8,6}$=2.1 Hz), 7.91 (1H, d, Quin-H_5, $^3J_{5,6}$=9.0 Hz), 7.49 (1H, d, Ar-H_6, $^4J_{6,4}$=1.8 Hz), 7.46 (1H, dd, Quin-H_6, $^3J_{6,5}$=9.0 Hz, $^4J_{6,8}$=2.1 Hz), 7.35–7.42 (1H, m, Ph), 7.24–7.31 (2H, m, Ar-H_3, Ar-H_4), 7.16–7.22 (2H, m, Ph), 7.03–7.10 (1H, m, Ph), 7.07 (1H, d, Quin-H_3, $^3J_{3,2}$=5.3 Hz), 6.85 (1H, s broad, NH), 3.56 (2H, s, CH_2), 2.45–2.49 (4H, m, N-CH_2), 1.72–1.77 ppm (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ=151.9 (Quin-C_2), 130.9 (Ar-C_3), 129.4 (d, Ph, $^3J_{CH,F}$=8.3 Hz), 128.9 (Quin-C_8), 126.1 (Quin-C_6), 125.2 (Ph), 123.3 (Ar-C_6), 121.2 (Quin-C_5), 120.4 (Ar-C_4), 116.6 (d, Ph, $^2J_{CH,F}$=21.6 Hz), 113.8 (d, Ph, $^2J_{CH,F}$=21.2 Hz), 102.6 (Quin-C_3), 57.2 (CH_2), 54.0 (2C, N-CH_2), 23.5 ppm (2C, CH_2); MS *m/z*: 432.0–434.0 $[M+H]^+$; HPLC (C_{18}, 10 min), $HPLC_{prep}$: 98%, t_R=4.1 min; HPLC (C_{18}, 40 min), $HPLC_{prep}$: 98%, t_R=18.0 min; HPLC (C_4, 40 min), $HPLC_{prep}$: >99%, t_R=17.3 min.

Biological evaluation

In vitro *P. falciparum* culture and drug assays. *P. falciparum* strains were maintained continuously in culture on human erythrocytes as described by Trager and Jensen.[30] In vitro antiplasmodial activity was determined by using a modification of the semi-automated micro-dilution technique of Desjardins et al.[31] *P. falciparum* CQ-sensitive (F32/Tanzania and Thai/Thailand) and CQ-resistant (PFB/Brazil, FcB1R/Colombia, and K1/Thailand) strains were used in sensitivity testing. Stock solutions of chloroquine diphosphate and test compounds were prepared in sterile distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and introduced to asynchronous parasite cultures (1%

parasitemia and 1% final hematocrit) 96-well plates for 24 h at 37 °C prior to the addition of [^{3}H]hypoxanthine (0.5 μCi per well, 1–5 Ci mmol^{-1}; Amersham, Les Ulis, France) for 24 h. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture (without drug) maintained on the same plate. Concentrations causing 50% inhibition (IC_{50}) or 90% inhibition (IC_{90}) were obtained from the drug concentration–response curve, and the results are expressed as the mean ±SD determined from several independent experiments. The DMSO concentration never exceeded 0.1% and did not inhibit parasite growth.

Cytotoxicity tests with MRC-5 cells. A human diploid embryonic lung cell line (MRC-5, Bio-Whittaker 72211D) was used to assess the cytotoxic effects on host cells. MRC-5 cells were seeded at 5000 cells per well. After 24 h, the cells were washed, and twofold dilutions of the drug were added in 200 μL standard culture medium (RPMI with 5% fetal calf serum). The final DMSO concentration in the culture remained <0.5%. The cultures were incubated with several concentrations of compounds (between 32 and 1.6 μM) at 37 °C in 5% CO_2/95% air for 7 days. Untreated cultures were included as controls. The cytotoxicity was determined with the colorimetric MTT assay[32] and scored as a percent decrease in absorption (λ = 540 nm) of treated cultures versus untreated control cultures.

Inhibition of β-hematin formation. The ability of a compound to inhibit β-hematin formation induced by lipids[33] was determined by using the method developed by Ayad et al.[34] A solution of hemin (700 μM) in NaOH (25 mM) (500 μL) was added to a suspension of 1-monooleoylglycerol (1 mM) in sodium acetate (90 mM) at pH 5 (500 μL). Drugs were added from stock solutions in DMSO (10 μL). Samples were incubated for 24 h at 37 °C. Controls contained an equal amount of DMSO. Following incubation, samples were centrifuged at 27000 *g* at 4 °C for 15 min. The precipitate of β-hematin was washed several times with sodium phosphate (10 mM, pH 7.4, containing 2.5% SDS) and vortexed for 10 min at 20 °C before re-pelleting until supernatant was colorless. Dissolution of β-hematin was achieved by the addition of 900 μL sodium phosphate (10 mM, pH 7.4, containing 2.5% SDS) and 50 μL NaOH (1 M). Concentration of β-hematin was calculated from absorbance at 405 nm. Experiments were carried out in duplicate.

In vivo antimalarial evaluation. Female IOPS-OF1 mice (Charles River, Cleon, France) weighing approximately 20 g were inoculated with *P. berghei* ANKA. The in vivo antimalarial activity was evaluated by the 4 day suppressive test described by Peters.[35] Drugs were dissolved in pure DMSO at a concentration of 10 mg mL^{-1}. The blood of an infected mouse (about 20% parasitemia) was diluted in physiological saline solution (0.9% NaCl) to a density of 10^8 infected red blood cells per mL. At day 0, the mice were inoculated intraperitoneally (i.p.) with 0.1 mL of the parasite suspension. Four hours later, the mice were injected i.p. with 0.1 mL drug solution. The injection was repeated daily for three consecutive days. Parasitemia was determined on thin blood smears stained with Giemsa starting from the day 5. Animals were euthanized after 32 days. For each dose, four animals were tested. For the control group, animals were injected with pure DMSO, and all other steps were identical to those for the treated groups. Experiments were done with conformity to local policy regarding ethical animal experimentation.

X-ray crystallography. Details of crystal structure determination and refinement for compounds **11b**, **20b**, and **22a** are given in table 2 of the Supporting Information. Data were collected on a Bruker SMART APEX diffractometer using graphite-monochromated $Mo_{K\alpha}$ radiation (k = 0.71073 Å). For this purpose the crystals were attached on cryoloops and the data were collected at room temperature (T = 297 K). The structures were refined with anisotropic thermal parameters. The hydrogen atoms were refined with a riding model and a mutual isotropic thermal parameter. The H atoms hydrogen bonded to N2 from each of the three compounds were found in a difference map. For structure solution and refinement, the software package SHELX-97 was used.[36,37] The drawings were created with the Diamond program.[38,39] CCDC 668821 (**11b**), CCDC 668820 (**20b**), and CCDC 668822 (**22a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

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$$VAR = \frac{1+\sum_{n=1}^{4}\sum_{i=1}^{n} 10^{pK_{a_i}-pH_v}}{1+\sum_{n=1}^{4}\sum_{i=1}^{n} 10^{pK_{a_i}-pH_o}}$$

for which $pH_v = pH$ inside the vacuole (assumed to be pH 5.0) and $pH_o =$ external pH (assumed to be pH 7.4). This equation is a derivation of the Henderson–Hasselbach equation, based on predicted values of drug pK_a according to previous works of Hawley et al.[13a] Values of pK_a were calculated by using ACD/pK_a DB software from Advanced Chemistry Development Inc., Toronto (Canada).

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