

Overcoming Drug Resistance to Heme-Targeted Antimalarials by Systematic Side Chain Variation of 7-Chloro-4-aminoquinolines

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Abstract: Systematic variation of the branching and basicity of the side chain of chloroquine yielded a series of new 7-chloro-4-aminoquinoline derivatives exhibiting high *in vitro* activity against four different strains of *P. falciparum*. Many of the compounds tested showed excellent potency against chloroquine sensitive and resistant strains. In particular **4b**, **5a**, **5b**, **5d**, **17a**, and **17b** were found to be significantly more potent than chloroquine against the resistant strains Dd2 and FCB.

Malaria remains the world's most widespread and devastating infectious disease, with approximately 300 million annual cases and more than 2 million casualties. Among the protozoan parasites of the genus *Plasmodium* causing malaria in humans, *Plasmodium falciparum* is the most lethal species. Since the discovery of the antimalarial potency of quinine and other cinchona alkaloids, many agents exhibiting a 4-substituted quinoline pharmacophore have been introduced. In particular, chloroquine (CQ^a), mefloquine, sontoquine, and amodiaquine have proved to be among the most effective antimalarial drugs (Figure 1).^{1–3} Aminoquinolines are known to form a complex with ferriprotoxoporphyrin IX (FPIX), which is generated in the food vacuole of the intraerythrocytic malaria parasite as a result of proteolysis of host hemoglobin (Hb), which serves as a major source of amino acids during the protozoan life stages within the infected red blood cell. Free FPIX is cytotoxic to *Plasmodium*, which therefore has developed a strategy to limit the amount of free FPIX, converting it into insoluble crystalline hemozoin.⁴ The drug–FPIX interactions inhibit conversion of hematin to hemozoin and hence its detoxification via crystallization, and the accumulation of significant concentrations of toxic FPIX–aminoquinoline adducts is believed to be ultimately

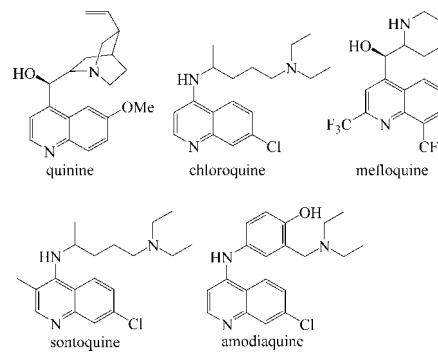


Figure 1. Structures of antimalarial aminoquinolines.

responsible for killing the parasite.^{5–9} It is widely accepted that the 4-aminoquinoline pharmacophore plays a crucial role in the complexation to FPIX resulting in inhibition of hemozoin formation and parasite growth,¹⁰ while the presence of a basic amino group in the side chain is generally considered essential for trapping high concentrations of the drug in the acidic food vacuole of the parasite.¹¹

To date, numerous isolates of *P. falciparum* have developed resistance against a majority of currently employed antimalarial drugs. In order to address the ever-increasing health impact of malaria, promising chloroquine resistant (CQR) reversal agents^{12,13} and new therapeutics¹⁴ including artemisinin and other endoperoxides have been introduced.^{15–20} However, the latter are less affordable in the most plagued tropical and subtropical regions and resistance to endoperoxide-derived antimalarials has already been reported.²¹ Arguably, quinine, chloroquine, and mefloquine are among the most successful antimalarial drugs ever used, and additional lead compounds with improved activity against CQR strains have been discovered via synthetic modifications of these structures.^{22–24} Importantly, 4-aminoquinolines carrying an aliphatic side chain are often well tolerated and afford excellent activity–toxicity profiles.²⁵ The evident need for safe, effective, and inexpensive antimalarials that are equally active against multiple species of *Plasmodia*, e.g., *P. falciparum* and *P. vivax*, has therefore directed increasing efforts to the design of new CQ analogues.

Since modifications of the 7-chloroquinoline ring, i.e., incorporation of other electron-withdrawing or electron-donating substituents such as amino and methoxy groups into the various positions in the quinoline ring, have generally proved detrimental to the antimalarial activity,^{26,27} a systematic variation of the side chain structure and basicity seems to be more promising. Although few comprehensive and methodical modifications of the CQ side chain have been reported to date, it has been established that both shortening and lengthening of the separation of the two aliphatic amino groups to 2–3 or 10–12 carbon atoms and the incorporation of a phenol moiety can lead to increased activity against CQR strains.^{28–31} Several studies revealed that introduction of a branched dialkylamino motif at the side chain terminus of CQ, e.g., replacement of the ethyl by isopropyl or *tert*-butyl groups, can furnish metabolically more stable antimalarials with enhanced lifetime and retained activity against drug resistant strains of *P. falciparum*.^{32,33}

We envisioned that incorporation of an increasing number of basic amino groups along with systematic structural variations (length and branching) of the aliphatic side chain attached to the potent 4-amino-7-chloroquinoline pharmacophore would

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^a Abbreviations: CQ, chloroquine; CQR, chloroquine resistant; CQS, chloroquine sensitive; Dd2, chloroquine resistant strain; FCB, chloroquine resistant strain; HB3 chloroquine sensitive strain; GCO3, chloroquine sensitive strain; FPIX, ferriprotoxoporphyrin IX; Hb, hemoglobin; *P. falciparum*, *Plasmodium falciparum*; IC₅₀, 50% inhibitory drug concentration; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; Pybop, benzotriazol-1-yl-oxytritypyrrolidinophosphonium hexafluorophosphate; CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine.

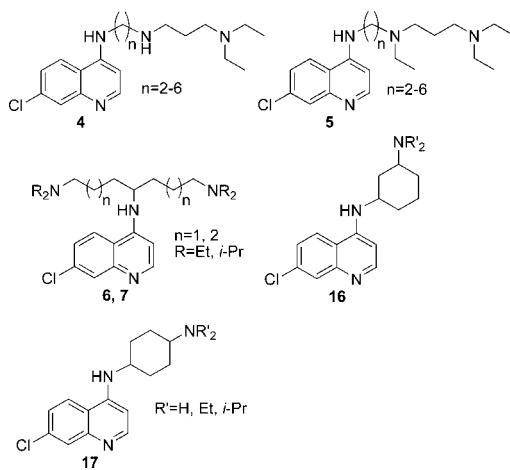


Figure 2. Structures of tribasic and dibasic 4-amino-7-chloroquinolines.

provide new candidates that overcome antimalarial drug resistance. The introduction of a highly branched tether between the two amino functions in CQ and the replacement of the metabolically unstable terminal diethylamino group by an isopropyl analogue were expected to enhance the lifetime of CQ analogues exhibiting retained activity against CQR strains. Herein, we report the synthesis and evaluation of the antimalarial activity of novel 4-amino-7-chloroquinolines carrying a branched or a linear side chain with two or three amino functions (Figure 2).

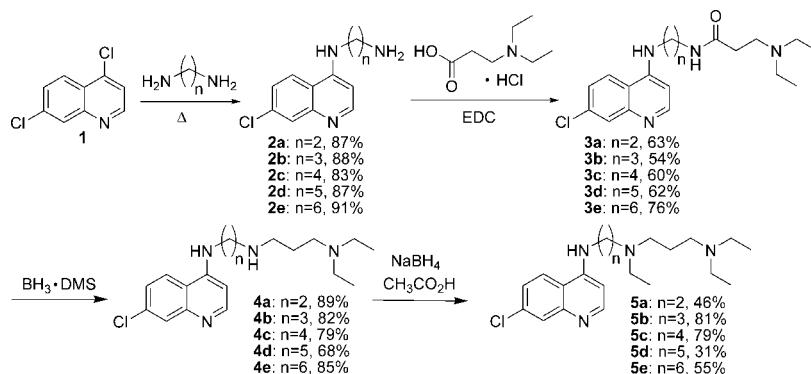
Our synthetic approach toward these heme-targeted antimalarials involved inexpensive materials and high-yielding steps in most cases. Amination of 4,7-dichloroquinoline, **1**, with commercially available α,ω -diaminoalkanes gave *N*-(7-chloro-4-quinolyl)-1, *n*-diaminoalkanes **2** in 83–91% yield (Scheme 1). Coupling of **2** with *N,N*-diethylamino-3-propionic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

hydrochloride (EDC) furnished amides **3**, which were reduced with borane–dimethyl sulfide to the corresponding series of secondary amines **4**. Finally, tertiary amines **5** were prepared by treatment of precursors **4** with sodium borohydride in glacial acetic acid.

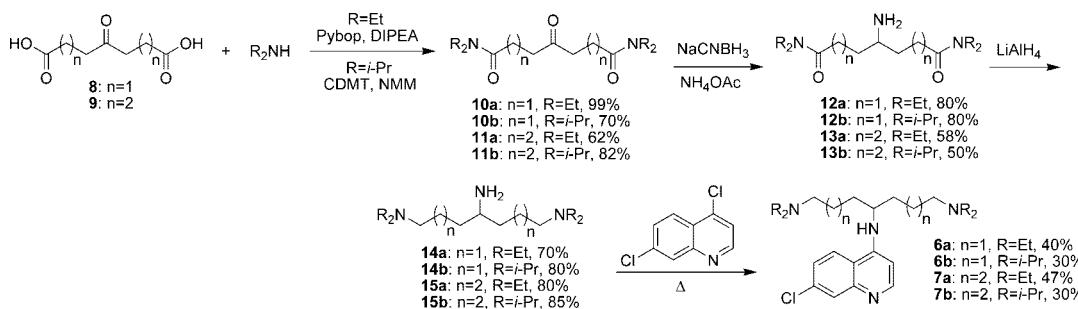
The symmetrically branched amines **6** and **7** were synthesized from 4-ketopimelic acid, **8**, and 5-oxoazelaic acid, **9** (Scheme 2). Screening of different coupling conditions revealed that Pybop and CDMT allow efficient amide formation with diethylamine and diisopropylamine, respectively. The corresponding α,ω -diamides **10** and **11** were thus obtained in 62–99% yield. Reductive amination of the ketone group using ammonium acetate and sodium cyanoborohydride and subsequent reduction of the terminal amides with lithium aluminum hydride gave triamines **14** and **15** in good yields. These amines were then employed in a carefully optimized nucleophilic aromatic substitution procedure using excess of **1** at high temperatures in a closed vessel to produce chloroquinolines **6** and **7**. The 1,3- and 1,4-diaminocyclohexane-derived chloroquinolines **16a**, **16b**, **17a**, and **17b** were prepared from 4,7-dichloroquinoline and a mixture of the *cis*- and *trans*-isomers of diaminocyclohexanes **18** and **19**. The nucleophilic halide displacements were followed by treatment of intermediates **20** and **21** with either NaBH₄ and acetic acid or acetone and NaBH(OAc)₃ (Scheme 3).

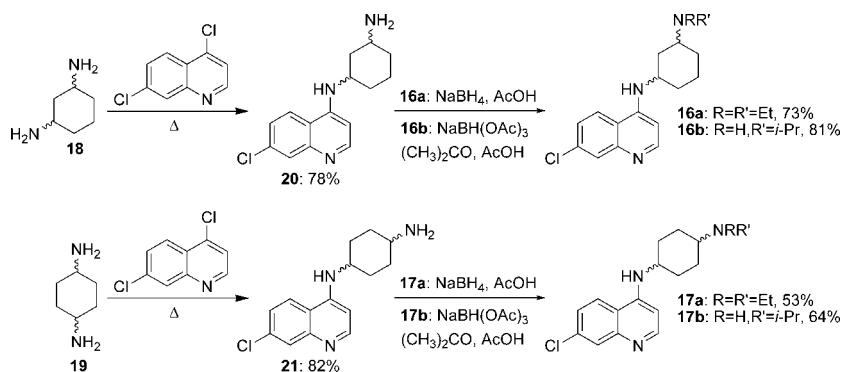
The antiplasmodial activity of tribasic compounds **4a–e**, **5a–e**, **6a,b**, and **7a,b** and the dibasic 1,3- and 1,4-diaminocyclohexane-derived chloroquinoline analogues **16a**, **16b**, **17a**, and **17b** was measured versus two CQS (HB3 and GCO3) and two CQR (Dd2 and FCB) strains using a standardized, inexpensive assay based on SYBR Green I intercalation that has recently been adopted and validated by several laboratories.^{34–36} The IC₅₀ values were calculated from experiments carried out in triplicate and compared to CQ (Table 1). Many of the aminoquinolines prepared for this study showed antimalarial activity versus HB3 and GCO3 similar to that of CQ, and we

Scheme 1. Synthesis of 4-Amino-7-chloroquinolines **4a–e** and **5a–e**



Scheme 2. Synthesis of Symmetrically Branched 4-Amino-7-chloroquinolines **6a,b** and **7a,b**



Scheme 3. Synthesis of 1,3- and 1,4-Diaminocyclohexane-Derived Chloroquinolines **16a,b** and **17a,b****Table 1.** Antiplasmodial Activity

compd	strain/experimental IC ₅₀ ^a (nM)					
	HB3	Dd2	SI ^b	GCO3	FCB	SI ^b
CQ	13.5	140	10.4	16.0	170	10.6
4a	29.2	129	4.43	118	146	1.24
4b	27.3	56.3	2.06	64.8	73.7	1.14
4c	72.5	170	2.34	139	189	1.36
4d	46.0	103	2.25	68.7	97.3	1.42
4e	82.8	269	3.25	129	216	1.67
5a	27.3	31.2	1.14	37.7	52.7	1.40
5b	21.2	28.1	1.33	27.6	49.1	1.78
5c	24.1	84.6	3.52	87.4	156	1.78
5d	15.7	43.4	2.76	55.2	66.8	1.21
5e	62.9	274	4.36	175	263	1.50
6a	187	128	0.68	96.6	186	1.92
6b	44.1	99.8	2.26	42.4	104	2.45
7a	716	882	1.23	517	1060	2.05
7b	1314	2550	1.94	1512	2225	1.47
16a	26.3	80.0	3.04	40.6	113	2.78
17a	25.5	51.8	2.03	32.0	57.6	1.80
16b	27.8	76.1	2.74	26.1	110	4.21
17b	31.3	75.7	2.42	19.5	66.0	3.89

^a The experimental IC₅₀ values are averages of two separate determinations each conducted in triplicate. ^b The selectivity index (SI) is the ratio of the IC₅₀ for the resistant versus the sensitive strain (Dd2/HB3, 4th column; FCB/GCO3, 7th column).

were pleased to find that several compounds were significantly more potent against the resistant strains Dd2 and FCB than CQ. Among the 18 compounds evaluated, 8 gave IC₅₀ values between 28.1 and 80.0 nM for Dd2 (CQ IC₅₀ = 140 nM) and 6 showed IC₅₀ values ranging from 49.1 to 73.7 nM for FCB (CQ IC₅₀ = 170 nM). Interestingly, the antimalarial activity of the linear tribasic aminoquinolines **4a–e** and **5a–e** proved to be generally superior over that of the tribasic compounds **6a**, **6b**, **7a**, and **7b** carrying a symmetrically branched side chain. Impressive antimalarial activity was also observed with the highly branched dibasic CQ analogues **16a**, **16b**, **17a**, and **17b**, but these compounds possess an inherently higher selectivity index (SI, the ratio of the IC₅₀ for a resistant versus a sensitive strain) than the linear tribasic aminoquinolines. Noteworthy, diastereomeric mixtures of **16a** and **17a** have previously been prepared by Drake et al. and Jensen later reported higher antimalarial activity against certain CQS and CQR strains relative to chloroquine.^{37–39} The selectivity index provides a quantitative measure of the antimalarial activity against CQR strains relative to that against sensitive strains and thus indicates promising drug discovery leads. The selectivity index of CQ is about 10, whereas all compounds tested have SI values between 0.68 and 4.43. In this regard, it is important that **5a** and **5b** combine high antimalarial activity against HB3 and GCO3 with very low SI values between 1.14 and 1.78. These new heme-targeted antiplasmodials thus show activity versus CQS strains

similar to that of CQ, and more importantly, they retain their potency against CQR strains. Comparison of the antimalarial activity of **5a** and **5b** with the results obtained for the tribasic aminoquinolines **4a** and **4b** suggests that the presence of a tertiary central amino group in this series is crucial for the activity against Dd2, GCO3, and FCB but not for HB3. Similarly, the potency of **5a** and **5b** versus Dd2, GCO3, and FCB diminishes when the chain length is increased. The impressive SI values of all compounds tested demonstrate that systematic variations of both the CQ side chain structure and basicity provide new venues to overcome antimalarial drug resistance. This can be combined with the introduction of a third basic amino function, which should further favor accumulation of the drug within the acidic food vacuole of the parasite. However, the relatively high IC₅₀ values of **6a**, **6b**, **7a**, and **7b** reveal that the basicity and structure of the side chain cannot be optimized independently.

In conclusion, we have prepared a series of new heme-targeted antimalarials by systematically varying the structure and basicity of the side chain attached to the 7-chloro-4-aminoquinoline pharmacophore of CQ. All 18 compounds tested show potent antimalarial activity against 4 different strains *in vitro* and can be synthesized from readily available, inexpensive starting materials through a few high-yielding steps. Comparison with CQ revealed that **4b**, **5a**, **5b**, **5d**, **16a**, **16b**, **17a**, and **17b** afford clearly superior activity against the CQ resistant strain Dd2, and **4b**, **5a**, **5b**, **5d**, **17a**, and **17b** proved significantly more potent against FCB. In particular, the tribasic 4-aminoquinolines **5a** and **5b** carrying a short linear side chain with two additional aliphatic tertiary amino functions are highly potent antimalarials and equally effective against both CQS and CQR strains. This study reveals that methodical variation of the side chain of chloroquine provides a promising entry toward affordable heme-targeted antimalarials that overcome the ever-increasing problem with worldwide drug resistance.

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Supporting Information Available: Synthetic procedures of all compounds tested and synthetic precursors, product characterization, ¹H and ¹³C NMR spectra, and HPLC chromatograms obtained by two orthogonal methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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