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Bis-alkylamine quindolone derivatives as new antimalarial leads

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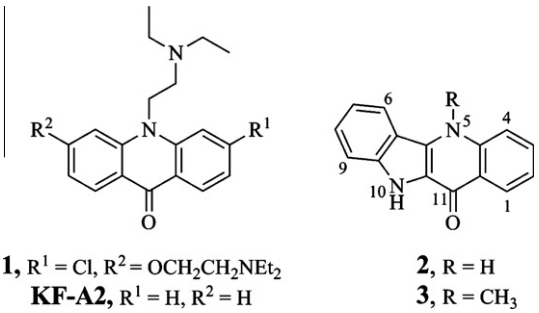
ABSTRACT

Quindolone derivatives, designed to target the malaria parasite digestive vacuole and heme detoxification pathway, have been synthesized by reaction with 2-chloro-*N*,*N*-diethylethanamine. This reaction gave N,O-, N,N- and O-alkylated products containing one or two basic side-chains. The compounds were evaluated for antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* W2 strain and for cytotoxicity in HepG2 A16 hepatic cells. By incorporating alkylamine side chains and chlorine atoms in the quindolone nucleus we transformed the inactive tetracyclic parent quindolones into moderate or highly active and selective antimalarial compounds. The most active and selective compound, **5c**, showed an IC_{50} = 51 nM for *P. falciparum* and a selectivity ratio of 98.

Malaria is one of the most widespread infectious diseases of our time due to the rapid emergence and spread of multidrug-resistant strains of *Plasmodium falciparum*, the mostly lethal of the malaria parasite species. Although attempts to develop a vaccine for malaria are ongoing, drugs remain a key component of control strategies. With continued spread of drug resistance, there is an urgent need for novel antimalarial drugs.[1,2]

During their erythrocytic stage malaria parasites feed on host hemoglobin, releasing toxic free heme, which is biocrystallized into hemozoin or malaria pigment, which is harmless to the parasite.[3,4]Heme detoxification remains one of the most attractive drug development targets, mainly due to the immutable nature of heme molecules.[5,6] Several chemotypes of antiplasmodial drugs, such as 4-aminoquinolines (e.g., chloroquine, CQ), xanthones, acridines and indoloquinolines are reported to act by enhancing free heme toxicity through inhibition of hemozoin formation.[5,7–10] Additionally, we showed that incorporation of a basic side chain at C-11 of the 5-methyl-10*H*-indolo[3,2-*b*]quinoline scaffold increased antiplasmodial activity about 10-fold, possibly by increasing drug accumulation inside the acidic digestive vacuole with a pH-dependent trapping mechanism.[11] Acridones have also been pursued as leads for new antimalarials. Haloalkoxyacridones were shown to be very potent and selective antiplasmodial compounds, although the mechanism of action of these acridone derivatives has been proposed to be mainly inhibition of the respiratory chain of parasite mitochondria.[12] However, after introducing a basic tertiary amine group, as in the case of **1** (Fig. 1), the acridone accumulates inside the digestive vacuole and kills the parasite by inhibition of hemozoin formation.[13]

Our research group recognized the antimalarial potential of indolo[3,2-*b*]quinolines and acridones. By merging the two scaffolds we obtained quindolone **2** (indolo[3,2-*b*]quinolin-11-one, Fig. 1). Quindolone and its *N*5-methyl derivative **3** (Fig. 1) were first isolated from the African medicinal plant *Cryptolepis sanguinolenta*.[10] Although **3** was inactive against chloroquine-resistant *P. falciparum* strain K1,[14] its tetracyclic aromatic structure and hydrogen-bond acceptor carbonyl group are believed to be structural features required for strong binding to heme or hemozoin



1, R^1 = Cl, R^2 = $OCH_2CH_2NEt_2$
KF-A2, R^1 = H, R^2 = H

2, R = H
3, R = CH_3

Figure 1. Structure of bis-alkylamine-acridone (**1**), quindolone (**2**), N5-methylquindolone (**3**) and acridone derivative (KF-A2).

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crystals,[6] providing the drug can reach its target inside the parasite digestive vacuole. Taking advantage of the three nucleophilic groups of quindolone, *N*-5, *N*-10 and *O*-11, we designed bis-alkylamine quindolone derivatives **4** and **5** (Scheme 1), aiming to target the parasite's digestive vacuole and the heme detoxification pathway. Here we describe the synthesis, in vitro antiplasmodial activity, cytotoxicity and heme binding affinity constants of these quindolone derivatives.

The synthetic route of quindolones **2** is depicted in Scheme 1 and follows the method previously described.[11] The bis-alkylated quindolone derivatives **4** and **5** and the monoalkylated derivative **6** were obtained after reaction of **2** with four equivalents of 2-chloro-*N,N*-diethylethanamine in the presence of a base and NaI, through a nucleophilic substitution reaction. The structures of quindolone derivatives **4**, **5** and **6** were established on the basis of bidimensional ^{1}H and ^{13}C heterocorrelation experiments (HMQC and HMBC), elemental analysis and melting point determination.[15] The positions of *N*-alkylamine side-chains for derivatives **4** and **5** were assigned on the basis of ^{1}H–^{13}C correlations between CH_2 protons of the side chain and quaternary carbons of the quindolone aromatic structure, observed on HMBC spectra and confirmed by Nuclear Overhauser Effect (NOE) experiments. For instance, the ^{1}H and ^{13}C NMR spectra of derivative **5a** (2-(11-(2-(diethylamino)ethoxy)-10*H*-indolo[3,2-*b*]quinolin-10-yl)-*N,N*-diethylethanamine) displayed signals corresponding to the introduction of two alkylamine side-chains in the quindolone nucleus. Also, the ^{13}C NMR showed signals corroborating the introduction of two side-chains. The disappearance of the carbonyl carbon signal of the starting quindolone resonating at 167.77 ppm, replaced by a phenoxy carbon signal at 144.58 ppm (C11) and a typical ether carbon signal (74.51 ppm), correlating in the HMQC with one deshielded triplet ^{1}H signal of the side chain, indicated the presence of an alkoxy chain at C-11. Further experiments revealed the attendance of NOE between the ^{1}H triplet signals of the CH_2 of the side-chains with the aromatic protons H-1 and H-9, corroborating alkylation in positions *O*-11 and *N*-10, respectively.

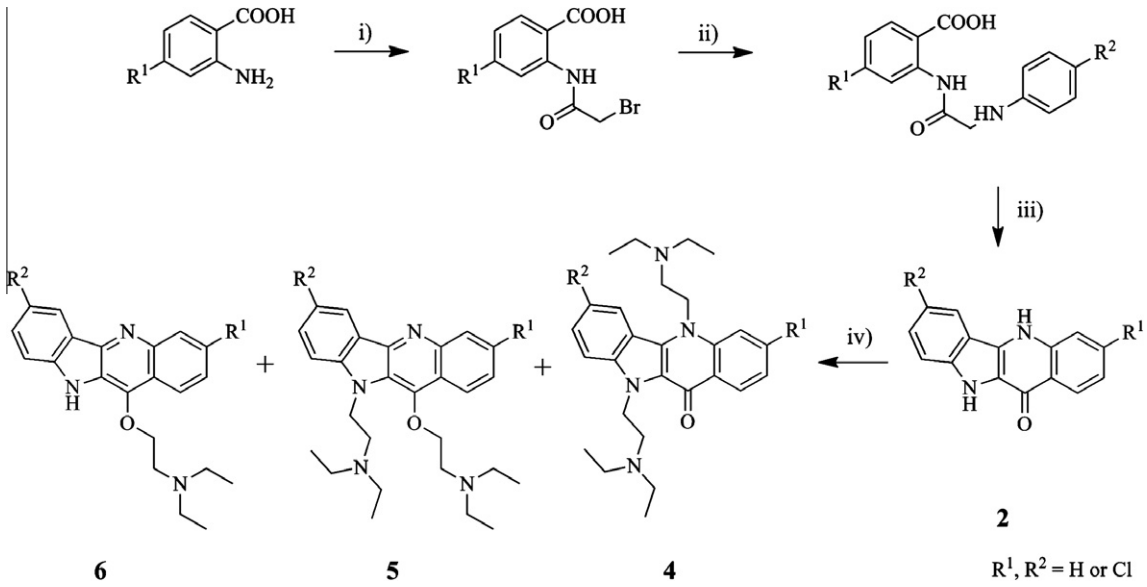
Several studies have considered the influence of base and solvent on the regioselectivity of O- versus N-alkylation in pyridones and quinolones.[16,17] Therefore, the alkylation reaction was attempted with different bases, namely, K_2CO_3, NaH, triethylamine and *N,N*-diisoproprylethylamine in dry acetone, tetrahydrofuran and dimethoxyethane. The best yields were achieved using K_2CO_3in dry acetone. In these conditions the major products were the bis-alkylated derivatives, with preference for the N,O-derivatives (**5**), with yields of 34–55% (Table 1). The formation of O-alkylated compounds as the main products in the alkylation reaction of quindolones **2** may be justified by the double-tautomeric resonance between the C-11 carbonyl and both *NH*-5, as in pyridinones,[16,18,19] and *NH*-10, as already discussed for natural quindolone **3**.[14,20]

Table 1
Alkylation yields of the quindolone derivatives (**4**–**6**)

Reagent	R^1, R^2	Alkylation yield (%)		
		N,N (**4**)	*N,O* (**5**)	*O* (**6**)
2a	H, H	23	55	17
2b	Cl, H	21	33	7
2c	Cl, Cl	19	34	5

It is also noteworthy that chlorine atoms, particularly in position 3 of the quindolone nucleus, deactivated O-alkylation, as yields of **5b**–**c** and **6b**–**c** were significantly lower than those of **5a** and **6a** (Table 1).

Quindolones **2** and derivatives **4**, **5** and **6** were evaluated for their antiplasmodial activity against *P. falciparum* chloroquine-resistant strain W2.[21] From the data in Table 2 it can be concluded that introduction of an alkylamine side chain is essential for antiplasmodial activity, as unsubstituted quindolones **2a**–**c** were inactive (IC_{50} 8 to >10 μM) and monoalkylated derivatives **6a**–**c** were only weakly (IC_{50} 2.6–1.3 μM) or moderately (IC_{50} of 0.55 μM) active, probably due to increased accumulation in the parasite digestive vacuole and affinity for the hemozoin crystal promoted by the tertiary amine group of the alkyl side chain.[5,6] Interestingly, monoalkylated quindolone derivative **6a** showed antiplasmodial activity at the same order of magnitude as that reported for the acridone KF-A2 (Fig. 1), which showed an IC_{50} of 7.5 μM for the CQ-resistant *P. falciparum* Dd2 strain.[22] Chlorine atoms at both sides of the tetracyclic aromatic structure also improved antiplasmodial activity, with the monohalogenated derivative **6b** two-times more active than **6a** and the dihalogenated derivative **6c** even more active.



Scheme 1. Quindolone **2** and derivatives **4**, **5** and **6** synthetic pathway. Reagent and conditions: (i) DMF/1,4-dioxane (1:1), bromoacetyl bromide, room temperature, overnight; (ii) aniline, DMF, 120 °C, 18–48 h; (iv) 2-chloro-*N,N*-diethylethanaminium, dry acetone, K_2CO_3 NaI, reflux, overnight.

Table 2
In vitro antiplasmodial activity (IC_{50}) against *P. falciparum* chloroquine-resistant strain W2, cytotoxicity to hepatic cell line HepG2 A16, selectivity ratio (SR) and association constants (K_{ass} in M^{-1}) for binding to hematin monomer at 25 °C in HEPES buffer pH 5.5 of quindolones **2** and their derivatives **4**, **5** and **6**

Compd	R^1	R^2	IC_{50} (±SD) *P. falciparum*W2 (nM)	IC_{50} (±SD) cytotoxicity HepG2 A16 (μM)	SR[a]	Haematin binding log K_{ass} (±SD)
CQ	—	—	138 (±15)	—	—	4.9 (±0.3)
2a	H	H	>10,000	21 (±2)	<2	4.7 (±0.3)
2b	Cl	H	8424 (±26)	—	—	5.0 (±0.5)
2c	Cl	Cl	>10,000	—	—	4.9 (±0.7)
4a	H	H	267 (±30)	4.33 (±0.02)	16	4.9 (±0.4)
4b	Cl	H	202 (±8)	7 (±1)	33	4.9 (±0.4)
4c	Cl	Cl	334 (±14)	11 (±2)	31	5.1 (±0.3)
5a	H	H	539 (±87)	7 (±1)	12	4.9 (±0.6)
5b	Cl	H	186 (±3)	8 (±2)	44	4.9 (±0.3)
5c	Cl	Cl	51 (±5)	5.0 (±0.9)	98	5.2 (±0.5)
6a	H	H	2638 (±22)	9 (±1)	3.6	5.0 (±0.5)
6b	Cl	H	1332 (±20)	—	—	5.1 (±0.6)
6c	Cl	Cl	550 (±40)	—	—	—

[a] Selectivity ratio ($IC_{50}^{HepG2A16}/IC_{50}^{W2}$).

The introduction of a second alkylamine side chain further increased antiplasmodial activity, with derivatives **4** and **5** showing IC_{50} values of 51–539 nM. Analyzing the influence of chlorine substitution on antiplasmodial activity of bis-alkylated derivatives **4** and **5**, it can be clearly seen that halogenation increases antiplasmodial activity of N,O-derivatives **5**, following the same pattern observed for **6**, whereas mono or di-halogenation of *N,N*-derivatives **4** has no effect on antiplasmodial activity. Improved antiplasmodial activity with introduction of chlorine atoms in quinolines and acridines has been noted previously, but the reason for this observation has not been clear.[9,23] As a consequence of the structure–activity relationships discussed above, the dihalogenated and bis-alkylated quindolone derivative **5c** emerges as the most active compound of the series, with an IC_{50} of 51 nM, slightly more active than chloroquine, and as active as the corresponding *N,O*-bis-alkylamineacridone **1** (IC_{50} = 77 nM for the Dd2 strain).[13]

Investigation of the proposed mechanism of antiplasmodial activity of quindolone derivatives was performed by determining equilibrium binding constants (K_{ass}) with hematin monomer (FPIX-OH), by UV–vis titration at pH 5.5.[24–27] Log K_{ass} shown in Table 2 were determined by fitting the experimental data to a binding model, with stoichiometry of one quindolone molecule to one FPIX-OH,[28] according to the binding stoichiometry determined with Job's method of continuous variations.[29–31].

Log K_{ass} values for quindolones **2** and derivatives **4**–**6** ranged from 4.7 to 5.2, comparable to the log K_{ass} values determined in our assay for chloroquine (4.9) and for the bis-alkylamine acridone **1** (4.85) in a similar assay.[13] From this experiment it can also be concluded that binding affinity of quindolone derivatives **4**–**6** to hematin monomer is only due to their aromatic tetracyclic structure, as their log K_{ass} values were not significantly different from those of parent quindolones **2**. Additionally, chlorine atoms in the aromatic structure of quindolone do not clearly affect π–π interactions with porphyrin, since differences between log K_{ass} of **2a** and **2b** or **2c** ($\leqslant$0.3) are less than the mean standard deviation (SD ≈0.5). Taken together, the results suggest that alkylaminequindolone derivatives **4**–**6** may share antiplasmodial mechanisms of action with chloroquine and acridone **1**, that is, inhibition of hemozoin formation, as far as compounds reach the target inside digestive vacuole.

Cytotoxicity was determined for the hepatic cell line HepG2 A16.[32] IC_{50} values for toxicity of quindolone **2a** and derivatives **4**–**6** ranged from 4.3 to 21 μM (Table 2). Introduction of alkylamine side chains slightly increased cytotoxicity, but no structure–cytotoxicity pattern was observed for quindolone derivatives **4**–**6**. The low cytotoxicity of alkylamine quindolone derivatives indicates selectivity against erythrocytic stages of malaria parasites. The selectivity ratio, determined as the cytotoxicity IC_{50}/antiplasmodial IC_{50} ratio, was higher than 10 for all bis-alkylated quindolone derivatives **4** and **5**. The most active compound **5c** was also the most selective: it was 100-fold more toxic to the parasite than to the human hepatic cells.

In conclusion, the introduction of two alkylamine side chains generally improves antiplasmodial activity, probably by increasing accumulation in the acidic digestive vacuole. On the other hand, substitution by chlorine atoms at positions 3 and 7 of the quindolone skeleton improves antiplasmodial activity for *N,O*-bis-alkylamine derivatives, but not for N,N-disubstituted derivatives, an interesting finding that deserves further investigation. Additionally, antiplasmodial activity of bis-alkylamine quindolone derivatives cannot be entirely explained by their affinity to hematin monomers. Taken together, these results indicate that the quindolone nucleus is a suitable scaffold for the design of active and selective compounds targeting the malaria parasite heme detoxification pathway and that bis-alkylaminequindolone derivatives are novel chemotypes with potential for development as antimalarial agents.

Acknowledgments

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15. *Synthesis of* **4a**, **5a**, *and* **6a**: To a solution of **2a** (40 mg, 0.17 mmol), K_2CO_3 (352.4 mg, 2.55 mmol, 15 equiv), NaI (101.9 mg, 0.68 mmol, 4 equiv) in dried acetone (15 mL) was added 2-chloro-N^1,N^1-diethylethanaminium chloride (117.0 mg, 0.68 mmol, 4 equiv) and refluxed overnight. At the end of time, solvent was removed at reduced pressure and the remain solid suspended in H_2O (30 mL). The aqueous solution was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic extracts, washed with water, brine, dried with anhydrous Na_2SO_4 and reduced to small volume. The crude mixture was purified by preparative thin layer chromatography (P-TLC) using as eluent CH_2Cl_2/MeOH (9:1). The compounds 5,10-bis(2-(diethylamino)ethyl)-5*H*-indolo[3,2-*b*]quinolin-11(10*H*)-one (**4a**, R_f 0.43), 2-(11-(2-(diethylamino)ethoxy)-10*H*-indolo[3,2-*b*]quinolin-10-yl)-*N*,*N*-diethylethanamine (**5a**, R_f 0.56) and 2-((10*H*-indolo[3,2-*b*]quinolin-11-yl)oxy)-*N*,*N*-diethylethanamine (**6a**, R_f 0.75), were isolated, as light yellow solids. Compounds were precipitated as hydrochlorides with HCl in Et_2O. 5,10-Bis(2-(diethylamino)ethyl)-5*H*-indolo[3,2-*b*]quinolin-11(10*H*)-one (**4a**) mp 228–231 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 8.69 (d, *J* = 8.3 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.70 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.54 (dd, *J* = 8.3, 7.6 Hz, 1H), 7.34 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.24 (dd, *J* = 8.4, 7.6 Hz, 1H), 5.00 (t, *J* = 8.0 Hz, 2H), 4.84 (t, *J* = 7.8 Hz, 2H), 3.03 (t, *J* = 8.0 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.71 (dq, *J* = 15.4, 7.1 Hz, 8H), 1.09 (dt, *J* = 15.4, 7.1 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C (ppm) 169.13, 139.70, 139.65, 131.38, 130.62, 127.32, 126.86, 124.89, 122.69, 122.60, 120.95, 119.52, 115.14, 114.11, 110.63, 53.17, 50.87, 47.69, 47.35, 43.10, 11.85. Anal. Calcd for $C_{27}H_{36}N_4O{\cdot}0.4HCl$: C, 72.52; H, 8.20; N, 12.53. Found: C, 72.26; H, 8.33; N, 12.27. 2-(11-(2-(Diethylamino)ethoxy)-10*H*-indolo[3,2-*b*]quinolin-10-yl)-*N*,*N*-diethylethanamine (**5a**) mp 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 8.55 (d, *J* = 7.7 Hz, 1H), 8.40 (d, *J* = 8.2 Hz, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 7.70 (dd, *J* = 8.5, 6.8 Hz, 1H), 7.66 (dd, *J* = 8.2, 7.4 Hz, 1H), 7.58 (dd, *J* = 8.2, 6.80 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.35 (dd, *J* = 7.7, 7.4 Hz, 1H), 4.73 (t, *J* = 7.70 Hz, 2H), 4.32 (t, *J* = 6.3 Hz, 2H), 3.10 (t, *J* = 6.3 Hz, 2H), 2.79 (t, *J* = 7.70 Hz, 2H), 2.70 (q, *J* = 7.1 Hz, 4H), 2.62 (q, *J* = 7.1 Hz, 4H), 1.13 (t, *J* = 7.1 Hz, 6H), 1.00 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C (ppm) 148.59, 145.83, 144.80, 144.58, 129.69, 129.31, 126.65, 124.87, 124.68, 122.46, 122.23, 122.08, 121.27, 119.86, 109.17, 74.51, 52.93, 51.71, 47.69, 47.62, 43.51, 12.06. Anal. Calcd for $C_{27}H_{38}N_4O{\cdot}4HCl$: C, 55.87; H, 7.29; N, 9.65. Found: C, 55.43; H, 7.39; N, 9.24. 2-((10*H*-Indolo[3,2-*b*]quinolin-11-yl)oxy)-*N*,*N*-diethylethanamine (**6a**): mp 193–195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 12.63 (s, NH), 8.56 (d, *J* = 7.8 Hz, 1H), 8.34 (d, *J* = 8.3 Hz, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 7.67 (dd, *J* = 8.6, 7.0 Hz, 1H), 7.60 (dd, *J* = 9.1, 7.1 Hz, 1H), 7.54 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.32 (dd, *J* = 7.8, 7.1 Hz, 1H), 4.58 (t, *J* = 7.5 Hz, 2H), 3.07 (t, *J* = 7.5 Hz, 2H), 2.90 (q, *J* = 7.2 Hz, 4H), 1.26 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 148.68, 145.54, 144.65, 143.52, 129.38, 128.95, 126.47, 124.23, 123.95, 122.58, 122.20, 121.48, 120.96, 119.37, 111.04, 73.99, 55.09, 48.51, 11.33. Anal. Calcd for $C_{21}H_{23}N_3O{\cdot}2.8HCl$: C, 57.91; H, 5.97; N, 9.65. Found: C, 57.56; H, 5.82; N, 9.83.
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32. HepG2 A16 hepatic cell line viability was determined based on MTT assay. Briefly, cells were seeded in a 96-well plate at a density of 1×10^4 cells/well. Different concentrations of each compound were incubated for 48 h under standard culture conditions. Concentrations inhibiting 50% of cell growth (IC_{50}) were determined from graphics of degree of cell injury over compound concentration.