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Original article

Synthesis and antimalarial activity of new analogues of amodiaquine

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Abstract

In order to determine the real significance of the 4′-phenolic group in the antimalarial activity and/or cytotoxicity of amodiaquine (AQ), analogues for which this functionality was shifted or modified were synthesized. Good in vitro antimalarial activity was obtained for compounds unable to form intramolecular hydrogen bond. Among the compounds synthesized, new amino derivative **5** displayed the greatest selectivity index towards the most CQ-resistant strain tested and was active in mice infected by *Plasmodium berghei.*

Keywords: Drug design; *Plasmodium falciparum*; Antimalarials; Amodiaquine; 4-Aminoquinolines

1. Introduction

Almost one-half of the world's population is exposed to the threat of malaria and the disease is responsible for two million deaths each year [1]. Chloroquine (CQ, Chart 1) was a mainstream drug in the fight against *Plasmodium falciparum*, but its efficacy is being eroded by the emergence of resistant parasites. The spread of CQ-resistance has prompted the re-examination of alternative antimalarials such as amodiaquine (AQ, Chart 1), an other 4-aminoquinoline which proved to be effective against CQ-resistant strains [2]. These early studies were confirmed by comparative trials of CQ and AQ for the treatment of acute, uncomplicated infections in Gambia, West and Central Africa and Nigeria. AQ was found to be superior to CQ, displaying lower parasitological and clinical failure rates [3–5]. However the use of AQ has been limited since the mid-1980s because of the occurrence of numerous cases of agranulocytosis and hepatotoxicity in adults taking the drug prophylactically [6,7]. AQ toxicity has been explained by the presence of its 4-hydroxyanilino moiety, which is believed to undergo extensive metabolization to its quinoneimine variant [8,9]. Formation of this reactive species in vivo and subsequent binding to cellular proteins and lipids could affect cellular function either directly or by immunological response [10,11]. This bio-activation was found to be accompanied by the expression of a drug-related antigen on the cell surface,

Abbreviations: AQ, amodiaquine; CQ, chloroquine; DIEA, diisopropylethylamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (thiazolyl blue).

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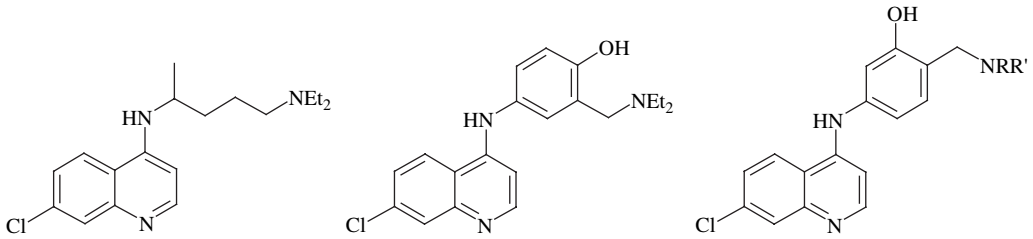


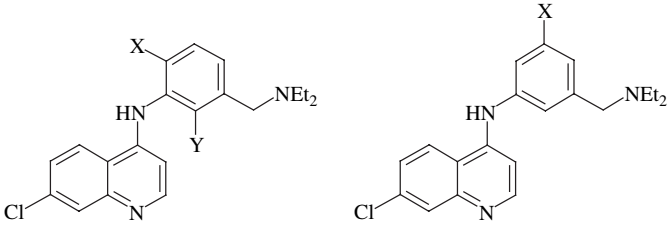
Chloroquine (CQ) **Amodiaquine (AQ)** **NRR' = NEt_2 : Isoquine (IsQ)**
NRR' = NHtBu : IsQ-tBu

Chart 1. Structure of chloroquine, amodiaquine and isoquine.

suggesting a type II hypersensitivity reaction [12] and causing the myelotoxicity of AQ [13]. Besides, activity of AQ is thought to be linked to an active conformation in which the internitrogen separation, i.e., between the quinoline nitrogen and the diethylamino nitrogen, is approximately 8.30 Å, like in CQ [14]. This distance is similar to that measured by X-ray crystallography between the central iron and the oxygens of the carboxylate groups of haem [15], the putative "receptor" of these 4-aminoquinolines. This active conformation is thought to be the result of an intramolecular hydrogen bond between the hydroxyl and the proton of the charged diethylamino function [16]. This hypothesis is at the basis of the design of fluoro analogs [17] and isoquine IsQ (Chart 1). IsQ analog IsQ–tBu is now planned for clinical trials [18].

Though resistance to AQ is developing, last WHO's guidelines for treatment still recommend the use of AQ in combination with artemisinin derivatives or if not available with sulfadoxine/pyrimethamine. As this antimalarial drug remains of fundamental therapeutic interest [19], the search for even more active analogs is of importance.

With the aim of studying the precise influence of the 4′-phenolic group of AQ for activity and toxicity, we report here the synthesis and antimalarial activity of the 2′-, 5′-, and 6′-hydroxyl isomers with the diethylaminomethyl moiety maintained in the 3′-position (compounds **1**, **2** and **4**, Chart 2). As previous work in our group showed the interest on amino substituent in 5′-position [20], two derivatives (**5** and **6**, Chart 2) were also synthesized. As a comparison, deoxo-AQ **3** [21] as well as the AQ isomer IsQ [22] (Charts 1 and 2), were also prepared and tested for their antimalarial activity and cytotoxicity upon MRC-5 cells.



1 X = H Y = OH
2 X = OH Y = H

3 X = H
4 X = OH
5 X = NH_2
6 X = NH-$(CH_2)_2$-piperazine

Chart 2. General structures of compounds **1**–**6**.

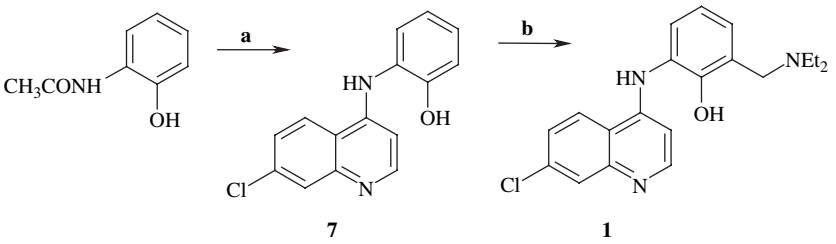
2. Chemistry

In the original synthesis of AQ, 4-hydroxyacetanilide was subjected to a Mannich reaction in order to introduce the diethylaminomethyl side chain *ortho* to the hydroxyl group [23]. Following hydrolysis of the amide function, the amine was condensed with 4,7-dichloroquinoline to give the desired compound. Compound **1** was synthesized using a similar method with inversion of the two steps (Scheme 1). Intermediate **7** was easily isolated by filtration in fairly good yield. Lower yield observed for compound **1** was due to side-reactions, such as formation of *para*-substituted product or double-Mannich reaction, which caused difficulties during purification. The same synthetic pathway could not be retained for other compounds since the orientation of substituents is different from that imposed by the Mannich reaction.

Compound **2** was prepared in four steps as described in Scheme 2. Reductive amination of 4-hydroxy-benzaldehyde with diethylamine gave intermediate **8**, which was transformed into the mono-nitro derivative **9** by using a solution of nitric acid in diluted sulfuric acid. The last two steps consisted of a reduction (iron powder in glacial acetic acid) of the nitro group, then condensation of the resultant amine with 4,7-dichloroquinoline which led, respectively, to compounds **10** and **2**. Low yields, which are not optimized, obtained from this synthetic pathway were due to degradation under reaction conditions.

Compound **3** was prepared in three steps as outlined in Scheme 3. Intermediate **11** was obtained by nucleophilic substitution of 3-nitrobenzylbromide with diethylamine. Nitro function was reduced with tin chloride, then aromatic substitution of 4,7-dichloroquinoline with resulting amine **12** provided compound **3**.

Synthesis of compound **4** was carried out in four steps (Scheme 4) with amino compound **5** as the intermediate. Oxidation of the previously described alcohol **13** [24], using the conditions described previously, led to the corresponding aldehyde **14**, which was directly transformed by reductive amination with diethylamine, to give compound **5**. The global yield of this one-pot synthesis was 30%. The last step comprised the reaction of the primary aromatic amine with nitrous acid in the presence of sulfuric acid as solvent, at 0 °C, which led to the diazonium salt. The latter gave the corresponding phenol **4** by thermal decomposition at 50 °C.



Scheme 1. Synthesis of compound **1**. Reagents: (a) HCl 20% then 4,7-dichloroquinoline, EtOH; (b) HCHO, $HNEt_2$, EtOH.

Compound **6** was prepared in three steps as described in Scheme 5. Substitution of *N*-(2-chloroethyl)piperidine with previously described intermediate **13** [24] gave alcohol **15**, which was oxidized to aldehyde using MnO_2 as reagent. Reductive amination of the aldehyde **16** with diethylamine gave compound **6**.

3. Biological results and discussion

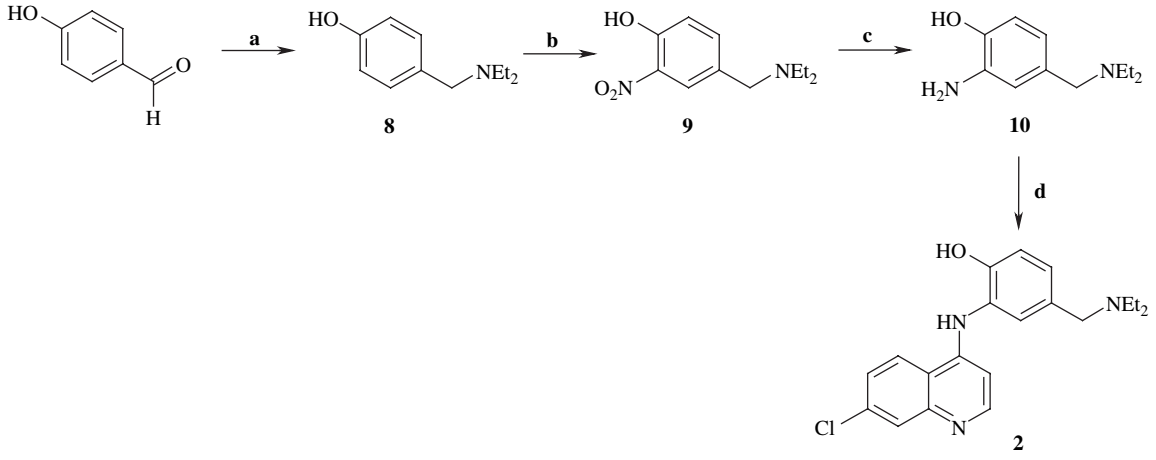
All the compounds were tested for their activity against a CQ-sensitive strain Thai (IC_{50} (CQ) = 14 nM), and two CQ-resistant strains FcB1R (IC_{50} (CQ) = 126 nM) and K1 (IC_{50} (CQ) = 183 nM). IC_{50} values for AQ were found to be 4.6 nM, 4.8 nM and 9.4 nM, respectively, against the three strains, therefore about a twice increase towards the most CQ-resistant strain (Table 1).

By comparison of their activities with those for AQ, compounds **1**–**6** can be ranged in four categories: (i) compound **3** (4′-deoxo-AQ) shows a slight decrease of activity compared with AQ or IsQ while IC_{50} remains stable upon all strains as for IsQ; (ii) compounds **2**, **5** and **6** show a slight decrease of activity upon Thai and FcB1R strains (IC_{50}s around 16 nM) and an obvious variability upon the most resistant-CQ strain (IC_{50}s between 26 and 32 nM); (iii) compound **4** is less active than the preceding ones and IC_{50}s increase with the CQ-resistance level of the strains; (iv) compound **1** (the 2′-isomer of AQ) is much less active than all the other derivatives (IC_{50}s around 100 nM upon Thai and FcB1R strains and superior to 250 nM upon K1 strain).

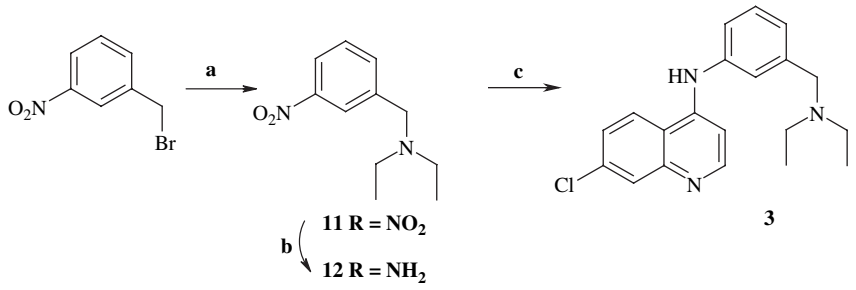
The cytotoxicity of the different compounds was evaluated upon human MRC-5 cells (Table 1). The MRC-5 cell is a diploid human fibroblast which is frequently used for cytotoxicity testing because of its higher sensitivity compared to some other cell lines. On the other hand, isomers **1**, **4** and the amino analog **5** were found to be twice less toxic than AQ while compound **3** has the same CC_{50} (concentration of drug causing 50% cytotoxicity) value as that of AQ. Secondary amino compound **6** was the most toxic one. AQ-isomers **1** (2′-isomer) and **2** (6′-isomer) could be oxidized as AQ. For dehydroxy-AQ **3** the possibility of its 4′-hydroxylation by metabolization in vivo cannot be ruled out, generating problems of toxicity as observed with AQ, agranulocytosis and hepatotoxicity. Comparison between 5′-AQ isomer **4** and 5′-amino analog **5**, shows that antimalarial activity is to the advantage of compound **5**.

Four out of the compounds synthesized revealed a selectivity index (CC_{50}/IC_{50} on K1 resistant strain) superior to that of CQ. Hence it was encouraging to consider amino compound **5** for further evaluation. Compound **5** was tested for its ability to inhibit haem polymerisation (Table 2). Results show that the compounds inhibit haem polymerization three times less than AQ and twice less than CQ.

Moreover as the basic phenol substituent of AQ is replaced in compound **5** by a neutral aromatic amino group, compound **5** is expected to be accumulated less in the acidic food vacuole of the parasite than AQ. Compound **5** was then tested in vivo



Scheme 2. Synthesis of compound **2**. Reagents: (a) $HNEt_2$, CH_2Cl_2 then $NaHB(OAc)_3$; (b) HNO_3, H_2SO_4, H_2O; (c) Fe, HCl, EtOH; (d) 4,7-dichloroquinoline, EtOH.



Scheme 3. Synthesis of compound **3**. Reagents: (a) $HNEt_2$, K_2CO_3, ACN, room temperature; (b) $SnCl_2$, HCl 1 M, THF, reflux; (c) 4,7-dichloroquinoline, HCl 1 M, ACN, reflux.

(Table 3) according to our typical procedure [25]. Compound **5** displayed a reasonable yet decreased activity when compared with CQ and AQ.

High antimalarial activity against CQ-resistant strains is described to be linked with the necessary formation of an intramolecular hydrogen bond between the hydroxyl function and the diethylammmonium group. When the latter is roughly well-positioned relative to the quinoline nitrogen [16] as in the case for IsQ, the level of activity is retained. However, activity is also maintained or slightly decreased, for compounds **2**–**5** where mobility of the diethylamino side chain is allowed. This result was already observed for compound **3** by Hawley [26]. It is only when a hydrogen-bonding interaction is likely to engage the diethylamino nitrogen at a much smaller distance from the quinoline nitrogen, as for compound **1**, that a significant decrease in antimalarial activity is observed.

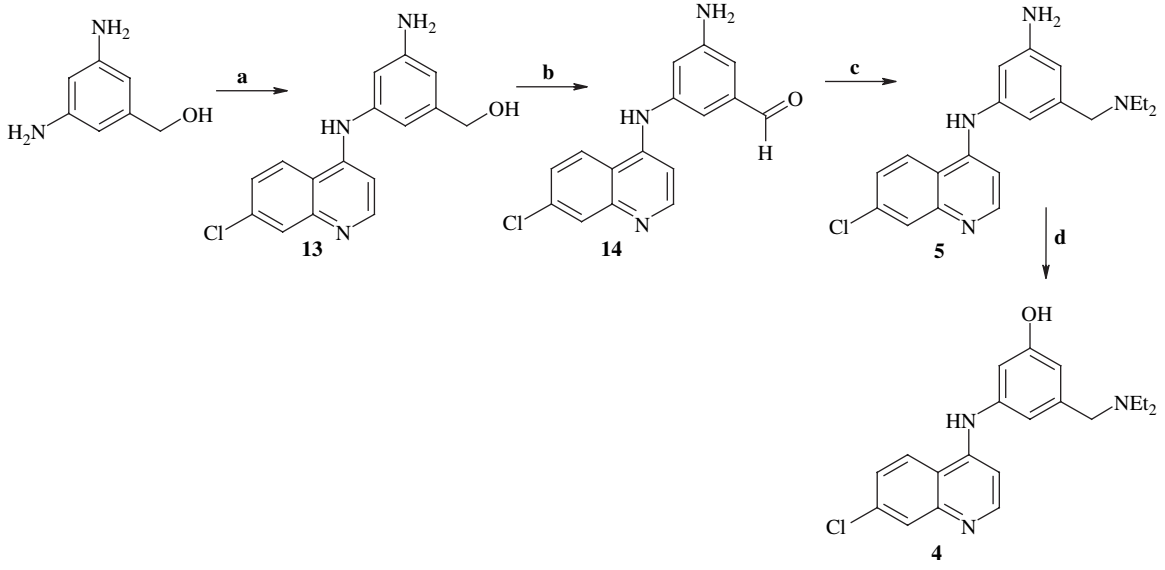
4. Conclusion

In conclusion, from among the AQ analogues studied, amino compound **5** displaying both a notable in vitro and in vivo antimalarial activity and the best selectivity index towards the most CQ-resistant strain K1, constitutes a good lead. Antimalarial activity of analog **6** is encouraging and the synthesis of compounds bearing other amino groups in 5′-position should be envisaged to decrease the cytotoxicity. Moreover, as the replacement of OH group in compound **4** by amino group in compounds **5** and **6** provides an increase in selectivity index, further work will comprise the synthesis of analogues presenting a variety of substituents in 4′-position.

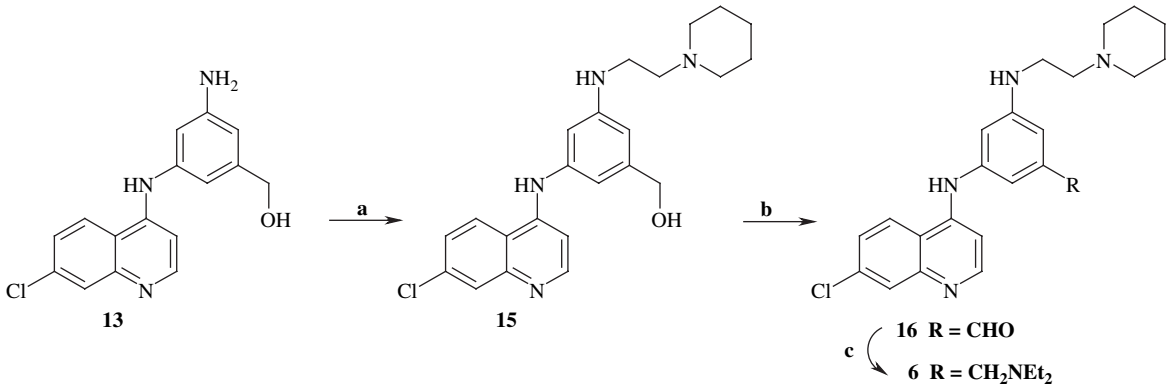
5. Materials and methods

5.1. Chemistry

All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. Chromatography was undertaken using silica gel 60 (230–400 mesh ASTM) from Macherey-Nagel. Thick-layer chromatography (TLC) was performed using silica gel from Merck, from which the compounds were extracted by the following solvent system: CH_2Cl_2/MeOH/NH_4OH, 80:20:1. All melting points were determined on a Büchi melting point apparatus and were



Scheme 4. Synthesis of compounds **4** and **5**. Reagents: (a) 4,7-dichloroquinoline, *N*-methylmorpholine, EtOH/$CHCl_3$, 55:5 [23]; (b) MnO_2, CH_2Cl_2; (c) $HNEt_2$, CH_2Cl_2 then $NaHB(OAc)_3$; (d) $NaNO_2$, H_2SO_4, 0 °C then 50 °C.



Scheme 5. Synthesis of compound **6**. Reagents: (a) *N*-(2-chloroethyl)piperidine, *n*-pentanol, reflux; (b) MnO_2, CH_2Cl_2; (c) $NaHB(OAc)_3$, CH_2Cl_2.

uncorrected. ^{1}H NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. Mass spectra were recorded on a MALDI-TOF Voyager-DE STR (Applied Biosystems, Palo Alto CA) with a trihydroxyacetophenone matrix. The purity of final compounds **1–6** was verified by high pressure liquid chromatography (HPLC) using C18 Vydac (C18V) column. The purity of intermediate compounds was verified using the same C18 V column (except compounds **11** and **12**) or C18 TSK gel super ODS (C18-TSK) column (intermediates **11** and **12**) (P_{HPLC}). Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in EtOH and injected through a 50 μL loop. The following eluent systems were used: A (H_2O/TFA, 100:0.05) and B (CH_3CN/H_2O/TFA, 80:20:0.05). HPLC retention times (t_R) were obtained, at flow rates of 1 mL/min, using the following conditions. For C18 V column : a gradient run from 100% eluent A during 5 min, then to 100% eluent B over the next 30 min, for C18-TSK column : a gradient run from 100% eluent A during 30 s, then to 100% eluent B over the next 8 min. 4,7-dichloroquinoline was obtained from Acros and other reagents from Acros, Aldrich, Avocado and Lancaster.

Table 1
In vitro antimalarial activity upon three *P. falciparum* strains, in vitro cytotoxicity of compounds **1–6** and selectivity index against K1 strain

Compound	IC_{50}[a] (nM)			SI[f]	CC_{50}[g] (μM) upon MRC-5 cells
	Thai	FcB1R	K1		
CQ	14.3 ± 2.4[e]	126 ± 26[e]	183 ± 35[e]	175	>32
AQ	4.6 ± 0.8[e]	4.8 ± 0.9[e]	9.4 ± 1.1[e]	1276	12
IsQ	4.3 ± 0.5[e]	5.4 ± 1.2[b]	7.3 ± 1.0[e]	1096	8
1	103.5 ± 14.3[e]	95.2 ± 21.8[b]	262 ± 20.5[d]	95	>25
2	15.2 ± 1.9[e]	15.4 ± 1.8[b]	32.6 ± 6.1[d]	583	19
3	13.2 ± 3.0[e]	15.8 ± 4.6[c]	17.1 ± 1.0[d]	731	12.5
4	25.3 ± 1.1[e]	35.2 ± 9.8[c]	42.8 ± 6.7[d]	584	>25
5	16.3 ± 2.1[e]	18.1 ± 0.8[b]	32.3 ± 4.2[d]	774	>25
6	15.6 ± 0.1[e]	19.1 ± 0.6[b]	26.5 ± 5.9[d]	152	4

[a] Parasites were considered resistant to CQ for IC_{50} >100 nM.
[b] $n = 3$.
[c] $n = 4$.
[d] $n = 5$.
[e] $n = 6$.
[f] Selectivity index towards K1 strain.
[g] CC_{50} is the concentration of drug causing 50% cytotoxicity, calculated on the basis of two experiments.

5.1.1. Synthesis of compound *1*

5.1.1.1. 2-(7-Chloroquinolin-4-ylamino)-phenol (7). A solution of 2-acetamidophenol (500 mg, 3.3 mmol) in 5 mL of HCl 20% was heated at reflux for 2 h. A solution of 4,7-dichloroquinoline (655 mg, 3.3 mmol) in 8 mL of EtOH was then added. After further stirring at reflux for 6 h, the mixture was cooled to 0 °C, filtered and the residue was washed by ether and dried under vacuum to yield the desired compound as a yellow solid (610 mg, 68% yield); R_f 0.60 (CH_2Cl_2/MeOH, 9:1); mp = 151 °C; HPLC (C18V) P_{HPLC} 100%, t_R 14.25 min; ^{1}H NMR (DMSO-d_6) δ 10.87 (s, 1H, OH), 10.24 (s, 1H, NH), 8.83 (d, $J = 9.2$ Hz, 1H, Quin-H_5), 8.48 (d, $J = 7.0$ Hz, 1H, Quin-H_2), 8.16 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 7.85 (dd, $J = 9.1$, 2.1 Hz, 1H, Quin-H_6), 7.31 (m, 2H, Ar-H_6, Ar-H_4), 7.15 (d, $J = 8.1$ Hz, 1H, Ar-H_3), 7.00 (t, $J = 8.1$ Hz, 1H, Ar-H_3), 6.32 (d, $J = 7.0$ Hz, 1H, Quin-H_3); *m/z* 271.5 ($M^+ + 1$).

5.1.1.2. 2-(7-Chloroquinolin-4-ylamino)-6-diethylaminomethyl-phenol (1). To a solution of compound **7** (610 mg, 2.3 mmol) in EtOH, were added formaldehyde 37% in water (5 equiv.) and diethylamine (1 equiv.). After stirring at reflux for 2 h, the mixture was concentrated and the residue was purified by TLC (CH_2Cl_2/MeOH/NH_4OH, 95:5:1) to yield the desired compound as a yellow solid (163 mg, 20% yield); R_f 0.75 (CH_2Cl_2/MeOH, 9:1); mp = 118 °C; HPLC (C18V) P_{HPLC} 99%, t_R 12.66 min; HPLC (C18N) P_{HPLC} 98%, t_R 13.60 min; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H, OH), 8.66 (s, 1H, NH), 8.44 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.33 (d, $J = 5.4$ Hz, 1H, Quin-H_2),

Table 2
Haem polymerisation inhibition

Compound	IC_{50} (μM)
CQ	70
AQ	48
5	153

Table 3
Antimalarial activity of compound **5** on *P. berghei* in mice

Compound	Dose (mg/kg)	Reduction (%) of parasitaemia on day 4	Excess MST[a] (%)
CQ	10	100	C[b]
AQ	10	100	C
5	20	96	63
5	40	100	C

[a] Excess MST is the change in the mean survival time of the treated mice, calculated by comparing the mean survival time of the control mice with the mean survival time of the treated mice.
[b] C for "cured" indicates mice surviving the infection and that can be termed cured definitively.

7.83 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 7.49 (dd, $J = 9.0, 2.3$ Hz, 1H, Quin-H_6), 7.10 (m, 2H, Ar-H_3, Ar-H_5), 6.81 (t, $J = 7.9$ Hz, 1H, Ar-H_4), 6.22 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 3.84 (s, 2H, CH_2), 2.58 (q, $J = 7.2$ Hz, 4H, CH_2CH_3), 1.02 (t, $J = 7.1$ Hz, 6H, CH_2CH_3); *m/z* 356.5 ($M^+ + 1$).

5.1.2. Synthesis of compound **2**

*5.1.2.1. 4-Diethylaminomethyl-phenol (**8**).* To a solution of 4-hydroxybenzaldehyde (1 g, 8.2 mmol) in 90 mL of dry CH_2Cl_2 were added diethylamine (2.54 mL, 24.6 mmol), after 2 h, $NaHB(OAc)_3$ (5.22 g, 32.8 mmol). After stirring the mixture at room temperature for 18 h, 10 mL of a solution of $NaHCO_3$ 1 M was added. The solvent was then evaporated and the residue was taken up in AcOEt. The reactive medium was then filtrated, the filtrate was concentrated and the residue purified by column chromatography (CH_2Cl_2/MeOH, 85:15) to yield compound **8** as a yellow solid of the sodium phenolate form (1.58 g, 96% yield); R_f 0.25 (CH_2Cl_2/MeOH, 8.5:1.5); mp = 79 °C; HPLC (C18V) P_{HPLC} 99%, t_R 9.07 min; ^{1}H NMR (DMSO-d_6) δ 6.99 (d, $J = 8.5$ Hz, 2H, Ar-H_3), 6.60 (d, $J = 8.5$ Hz, 2H, Ar-H_2), 3.32 (s, 2H, CH_2), 2.33 (q, $J = 7.2$ Hz, 4H, CH_2CH_3), 0.87 (t, $J = 7.1$ Hz, 6H, CH_2CH_3).

*5.1.2.2. 4-Diethylaminomethyl-2-nitrophenol (**9**).* To a solution of compound **8** (2.5 g, 12.5 mmol) in 5 mL of H_2SO_4, was added dropwise, at 0 °C, HNO_3 (150 μL, 2.5 mmol). After stirring at 0 °C for 30 min, the mixture was neutralized, at 0 °C, with Na_2CO_3, then concentrated. The residue was taken up in AcOEt, filtered and purified by column chromatography (CH_2Cl_2/MeOH, 90:10) to yield compound **9** as a yellow solid (550 mg, 20% yield); R_f 0.85 (CH_2Cl_2/MeOH, 8.5:1.5); mp = 106 °C; HPLC (C18V) P_{HPLC} 98%, t_R 10.03 min; ^{1}H NMR (DMSO-d_6) δ 7.79 (d, $J = 2.1$ Hz, 1H, Ar-H_3), 7.46 (dd, $J = 8.6$, 2.2 Hz, 1H, Ar-H_5), 7.06 (d, $J = 8.5$ Hz, 1H, Ar-H_6), 3.50 (s, 2H, CH_2), 2.47 (q, $J = 7.1$ Hz, 4H, CH_2CH_3), 0.97 (t, $J = 7.1$ Hz, 6H, CH_2CH_3); *m/z* 225 ($M^+ + 1$).

*5.1.2.3. 3-Amino-4-diethylaminomethyl-phenol (**10**).* To a solution of compound **9** (500 mg, 2.2 mmol) in 25 mL of a EtOH/H_2O, 1:1 mixture, were added iron powder (375 mg, 6.7 mmol) and concentrated chlorhydric acid (560 μL, 6.7 mmol). After stirring at reflux for 1 h, the mixture was filtered on celite, the residue washed with 3 × 20 mL of MeOH and the filtrate evaporated. The residue was then purified by TLC (CH_2Cl_2/MeOH, 80:20) to yield compound **10** as a brown oil (395 mg, 91% yield); R_f 0.35 (CH_2Cl_2/MeOH, 8.5:1.5); HPLC (C18V) P_{HPLC} 95%, t_R 5.07 min; ^{1}H NMR (DMSO-d_6) δ 9.45 (broad s, 1H, OH), 6.71–6.68 (m, 2H, Ar-H_3, Ar-H_6), 6.59 (dd, $J = 1.9$, 8.0 Hz, 1H, Ar-H_5), 4.68 (broad s, 2H, NH_2), 3.98 (s, 2H, CH_2), 2.88 (q, $J = 7.1$ Hz, 4H, CH_2CH_3), 1.21 (t, $J = 7.1$ Hz, 6H, CH_2CH_3); *m/z* 195 ($M^+ + 1$).

*5.1.2.4. 3-(7-Chloroquinolin-4-ylamino)-4-diethylamino-methyl-phenol (**2**).* To a solution of compound **10** (240 mg, 1.2 mmol) in 20 mL of EtOH, was added 4,7-dichloroquinoline (250 mg, 1.2 mmol) and the medium was alcanalized until pH 5.5. After stirring at reflux for 8 h, the mixture was concentrated and the residue purified by TLC (CH_2Cl_2/MeOH, 70:30) to yield compound **2** as a yellow solid (30 mg, 7% yield); R_f 0.40 (CH_2Cl_2/MeOH, 7:3); HPLC (C18V) P_{HPLC} 99%, t_R 11.22 min; ^{1}H NMR (DMSO-d_6) δ 9.47 (s, 1H, OH), 8.65 (s, 1H, NH), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.32 (d, $J = 5.4$ Hz, 1H, Quin-H_2), 7.82 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 7.48 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 7.12 (d, $J = 1.9$ Hz, 1H, Ar-H_3), 7.03 (dd, $J = 8.2$, 2.0 Hz, 1H, Ar-H_5), 6.90 (d, $J = 8.2$ Hz, 1H, Ar-H_6), 6.18 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 3.42 (s, 2H, CH_2), 2.39 (q, $J = 7.0$ Hz, 4H, CH_2CH_3), 0.93 (t, $J = 7.1$ Hz, 6H, CH_2CH_3); *m/z* 356.5 ($M^+ + 1$).

5.1.3. Synthesis of compound **3**

*5.1.3.1. Diethyl-(3-nitro-benzyl)-amine (**11**).* To a solution of 3-nitrobenzylbromide (200 mg, 0.93 mmol) in 25 mL of ACN, were added K_2CO_3 (640 mg, 4.6 mmol) and after 20 min stirring diethylamine (115 mL, 1.1 mmol). After stirring at room temperature for 8 h, the mixture was filtrated and concentrated. 50 mL of saturated solution $NaHCO_3$ was added and the aqueous layer extracted by 2 × 50 mL of CH_2Cl_2. The organic layers were then combined, dried over $MgSO_4$, the solvent was evaporated and the residue purified by TLC (cyclohexane/EtOAc/NH_4OH, 8:2:0.1) to yield compound **11** as a yellow oil (187 mg, 97% yield); R_f 0.65 (cyclohexane/EtOAc/NH_4OH, 8:2:0.1); HPLC (C18V) P_{HPLC} 99%, t_R 2.95 min; ^{1}H NMR ($CDCl_3$) δ 8.22 (t, $J = 1.8$ Hz, 1H, Ar-H_2), 8.06 (m, 1H, Ar-H_4), 8.22 (dt, $J = 8.0$, 1.8 Hz, 1H, Ar-H_6), 7.47 (t, $J = 8.0$ Hz, 1H, Ar-H_5), 3.66 (s, 2H, CH_2), 2.54 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 1.05 (t, $J = 7.0$ Hz, 3H, CH_2CH_3); *m/z* 209.2($M^+ + 1$).

*5.1.3.2. Diethyl-(3-amino-benzyl)-amine (**12**).* To a solution of nitro compound **11** (195 mg, 0.94 mmol) in 20 mL of THF, was added a solution of tin chloride (711 mg, 3.7 mmol) in 5 mL of THF with HCl 1 M (2.8 mmol). After stirring at reflux for 5 h, the mixture was concentrated, alkalinized with $NaHCO_3$ (pH 8) and the aqueous layer extracted by 5 × 50 mL of CH_2Cl_2. The organic layers were then combined, dried over $MgSO_4$, the solvent was evaporated and

the residue purified by TLC (CH_2Cl_2/MeOH/NH_4OH, 9.5:0.5:0.2) to yield compound **12** as a yellow oil (111 mg, 67% yield); R_f 0.6 (CH_2Cl_2/MeOH/NH_4OH, 9.5:0.5:0.2), HPLC (C18V) P_{HPLC} 95%, t_R 2.17 min; ^{1}H NMR ($CDCl_3$) δ 7.06 (t, $J = 8.0$ Hz, 1H, Ar-H_5), 6.70 (m, 2H, Ar-H_2, Ar-H_4), 6.53 (dt, $J = 8.0$, 1.8 Hz, 1H, Ar-H_6), 3.82 (broad s, 2H, NH_2), 3.49 (s, 2H, CH_2), 2.54 (q, $J = 7.0$ Hz, 2H, CH_2CH$_3$), 1.04 (t, $J = 7.0$ Hz, 3H, CH_2CH_3); *m/z* 179.2($M^+ + 1$).

*5.1.3.3. (7-Chloro-quinolin-4-yl)-(3-diethylaminomethyl-phenyl)-amine (**3**).* A solution of amine **12** (111 mg, 0.6 mmol) in 10 mL of ACN was added a solution of 4,7-dichloroquinoline (124 mg, 0.6 mmol) in 5 mL of ACN and 0.6 mL of HCl 1 M. After further stirring at reflux overnight, the mixture was concentrated and purified by TLC (CH_2Cl_2/MeOH/NH_4OH, 9.5:0.5:0.2) to yield compound **3** as a yellow solid (188 mg, 88% yield); R_f 0.8 (CH_2Cl_2/MeOH/NH_4OH, 9.5:0.5:0.2); mp = 142 °C; HPLC (C18V) P_{HPLC} 99%, t_R 12.84 min; ^{1}H NMR (MeOD) δ 8.26 (d, $J = 5.6$ Hz, 1H, Quin-H_2), 8.17 (d, $J = 9.0$ Hz, 1H, Quin-H_5), 7.75 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 7.38 (dd, $J = 9.0$, 2.1 Hz, 1H, Quin-H_6), 7.30 (t, $J = 7.8$ Hz, 1H, Ar-H_5), 7.25 (m,1H, Ar-H_2), 7.18 (d, $J = 7.8$ Hz, 1H, Ar-H_4), 7.08 (d, $J = 7.5$ Hz, 1H, Ar-H_6), 6.83 (d, $J = 5.6$ Hz, 1H, Quin-H_3), 3.57 (s, 2H, CH_2), 2.52 (q, $J = 7.0$ Hz, 2H, CH_2CH$_3$), 1.00 (t, $J = 7.0$ Hz, 3H, CH_2CH_3); *m/z* 340.2–342.2 ($M^+ + 1$).

*5.1.4. Synthesis of compounds **4** and **5***

*5.1.4.1. N-(7-Chloroquinolin-4-yl)-5-diethylaminomethyl-1,3-phenylenediamine (**5**).* To a solution of compound **13** [24] (300 mg, 1 mmol) in 15 mL of a CH_2Cl_2/DMF, 13:2 mixture, were added DIEA (175 μL, 1 mmol) and MnO_2 (1.3 g, 15 mmol). After stirring at room temperature for 18 h, the mixture was filtered on celite and the residue washed with 20 mL of CH_2Cl_2. To the resulting mixture containing the aldehyde **14** were added diethylamine (155 μL, 1.5 mmol) and, after 1 h, $NaHB(OAc)_3$ (424 mg, 2 mmol). After stirring the mixture at room temperature for 18 h, a solution of $NaHCO_3$ 1 M was added. Following further stirring of the mixture for 15 min, the layers were separated and the aqueous layer was washed twice with CH_2Cl_2. The organic layers were then combined, washed with brine, dried over $MgSO_4$ then the solvent was evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 80:20) to yield compound **5** as a yellow solid (110 mg, 31% yield); R_f 0.15 (CH_2Cl_2/MeOH, 8:2); mp = 61 °C; HPLC (C18V) P_{HPLC} 99%, t_R 11.33 min; ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H, NH), 8.49–8.43 (m, 2H, Quin-H_2, Quin-H_5), 7.85 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 7.51 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 6.89 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 6.48–6.45–6.37 (3 s, 3H, Ar-H), 5.19 (s, 2H, NH_2), 3.46 (s, 2H, CH_2), 2.53 (q, $J = 7.0$ Hz, 4H, CH_2CH_3), 1.00 (t, $J = 6.9$ Hz, 6H, CH_2CH_3); *m/z* 355.5 ($M^+ + 1$).

*5.1.4.2. 3-(7-Chloroquinolin-4-ylamino)-5-diethylaminomethyl phenol (**4**).* To a solution of compound **5** (100 mg, 0.28 mmol) in 5 mL of H_2SO_4 5%, was added, at 0 °C, a solution of $NaNO_2$ (23 mg, 0.33 mmol) in 2 mL of water. After stirring the mixture at 0 °C for 20 min, a urea crystal is added and following further stirring at 30 °C for an additional 15 min, a solution of $NaHCO_3$ 5% was added until neutralisation and the aqueous layer extracted by 2 × 30 mL of CH_2Cl_2. The organic layers were then combined, dried over $MgSO_4$, the solvent was evaporated and the residue purified by TLC (CH_2Cl_2/MeOH/NH_4OH, 90:10:1) to yield compound **4** as a yellow solid (40 mg, 40% yield); R_f 0.75 (CH_2Cl_2/MeOH, 7.5:2.5); mp = 77 °C; HPLC (C18V) P_{HPLC} 99%, t_R 11.60 min; ^{1}H NMR (DMSO-d_6) δ 9.52 (s, 1H, OH), 9.01 (s, 1H, NH), 8.46 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 8.41 (d, $J = 9.2$ Hz, 1H, Quin-H_5), 7.88 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 7.55 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 6.96 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 6.80–6.66–6.57 (3 s, 3H, Ar-H), 3.48 (s, 2H, CH_2), 2.57 (q, $J = 7.0$ Hz, 4H, CH_2CH_3), 1.02 (t, $J = 7.3$ Hz, 6H, CH_2CH_3); *m/z* 355.5 ($M^+ + 1$).

*5.1.5. Synthesis of compound **6***

*5.1.5.1. [3-(7-Chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-ethylamino)-phenyl]-methanol (**15**).* To a solution of compound **13** (300 mg, 1 mmol) in 10 mL of *n*-pentanol, was added *N*-(2-chloroethyl)piperidine hydrochloride (185 mg, 1 mmol) and *N*-ethylpiperidine (410 μL, 3 mmol). After stirring at reflux for 24 h, the mixture was concentrated and the residue purified by TLC (CH_2Cl_2/MeOH/NH_4OH, 80:20:1) to yield compound **14** as a yellow solid (240 mg, 57% yield); R_f 0.25 (CH_2Cl_2/MeOH, 8:2); HPLC (C18V) $P_{HPLC} > 99\%$, t_R 12.89 min; ^{1}H NMR (DMSO-d_6) δ 8.9 (s, 1H, NH), 8.40–8.43 (m, 2H, Quin-H_2, Quin-H_5), 7.85 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 7.52 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 6.92 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 6.51–6.42–6.37 (3 s, 3H, Ar-H), 5.53 (broad s, 1H, NH), 5.09 (broad s, 1H, OH), 4.49 (s, 2H, CH_2OH), 3.12 (m, 2H, CH_2NH), 2.46 (m, 2H, CH_2–CH_2NH), 2.39 (m, 4H, piperaz-H), 1.49 (m, 4H, piperaz-H), 1.39 (m, 2H, piperaz-H), *m/z* 411.3 ($M^+ + 1$).

*5.1.5.2. [3-(7-Chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-ethylamino)-phenyl]-methanal (**16**).* To a solution of compound **15** (500 mg, 1.2 mmol) in 40 mL of CH_2Cl_2, was added MnO_2 (1.59 g, 18.3 mmol). After stirring at room temperature for 18 h, the mixture was filtered on celite and the residue was washed with MeOH. The filtrate was then concentrated and the residue purified by TLC (CH_2Cl_2/MeOH, 85:15) to yield compound **15** as a yellow solid (140 mg, 30% yield); R_f 0.50 (CH_2Cl_2/MeOH, 85:15); HPLC (C18V) P_{HPLC} 99%, t_R 13.73 min; ^{1}H NMR (DMSO-d_6) δ 10.19 (s, 1H, CHO), 9.60 (broad s, 1H, NH), 8.76 (m, 2H, Quin-H_2, Quin-H_5), 8.18 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 7.84 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 7.31 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 7.41–7.25–7.22 (3s, 3H, Ar-H), 6.85 (s, 1H, NH), 3.30 (m, 2H, CH_2NH), 2.78 (m, 6H, CH_2–CH_2NH, piperaz-H), 2.02 (m, 4H, piperaz-H), 1.49 (m, 2H, piperaz-H), *m/z* 409.2 ($M^+ + 1$).

*5.1.5.3. N-(7-Chloroquinolin-4-yl)-5-diethylaminomethyl-N′-piperidin-1-ylethyl-1,3-phenylenediamine (**6**).* To a solution

of aldehyde **16** (135 mg, 0.33 mmol) in 10 mL of CH_2Cl_2, were added diethylamine (170 μL, 1.65 mmol.) and, after 1 h, $NaHB(OAc)_3$ (140 mg, 0.66 mmol.). After stirring the mixture at room temperature for 18 h, a solution of $NaHCO_3$ 1 M was added. Following further stirring of the mixture for 15 min, the layers were separated and the aqueous layer was washed twice with CH_2Cl_2. The organic layers were then combined, washed with brine, dried over $MgSO_4$ then the solvent was evaporated and the residue purified by (CH_2Cl_2/MeOH/NH_4OH, 90:10:1) to yield compound **6** as a yellow solid (70 mg, 46% yield); R_f 0.2 (CH_2Cl_2/MeOH, 8:2); mp = 51 °C; HPLC (C18V) P_{HPLC} 98%, t_R 12.76 min; ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H, NH), 8.40 (m, 2H, Quin-H_2, Quin-H_5), 7.83 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 7.50 (dd, $J = 9.0$, 2.2 Hz, 1H, Quin-H_6), 6.89 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 6.49–6.40–6.36 (3 s, 3H, Ar-H), 5.50 (broad s, 1H, NH_2), 3.39 (s, 2H, CH_2), 3.07 (m, 2H, CH_2NH), 2.49 (m, 6H, CH_2–CH_2NH), 2.44 (q, $J = 7.1$ Hz, 4H, CH_2CH$_3$), 2.34 (m, 4H, piperaz-H), 1.47 (m, 4H, piperaz-H), 1.35 (m, 2H, piperaz-H), 0.95 (t, $J = 7.1$ Hz, 6H, CH_2CH_3), *m/z* 466.2 ($M^+ + 1$).

5.2. Biological evaluation

5.2.1. 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 [27]. In vitro antiplasmodial activity was determined using a modification of the semi-automated microdilution technique of Desjardins [28]. *P. falciparum* CQ-sensitive (Thai/Thailand) and CQ-resistant (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 (0.5% parasitaemia and 1% final hematocrite) on plates comprising 96-wells for 24 h at 37 °C prior to the addition of 0.5 μCi of [^{3}H]hypoxanthine (1–5 Ci/mmol; Amersham, Les Ulis, France) per well, for 24 h. After freezing and thawing, the cells were harvested from each well onto glass fiber filters and the dried filters were counted in a scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated into the parasite nucleic acids with that in the control culture (without drug) maintained on the same plate. The concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration–response curve and the results were expressed as the mean ± the standard deviations determined from several independent experiments. The DMSO concentration never exceeded 0.1% and did not inhibit the parasite growth.

5.2.2. Cytotoxicity test upon MRC-5 cells

A human diploid embryonic lung cell line (MRC-5, Bio-Whittaker 72211D) was used to assess the cytotoxic effects towards host cells. MRC-5 cells were seeded at 5000 cells per well. After 24 h, the cells were washed and two-fold dilutions of the drug were added in 200 μL standard culture medium (RPMI + 5% FCS). The final DMSO concentration in the culture remained below 0.5%. The mammalian cell line was 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 using the colorimetric MTT assay [29] and scored as a % reduction of absorption at 540 nm of treated cultures versus untreated control cultures.

5.2.3. Heam polymerization inhibition

The ability of a compound to inhibit haem polymerization induced by lipids [30] was determined using the methods developed by Ayad [31]. Hemin and 1-monooleoylglycerol were purchased from Sigma. Experiments were carried out in duplicate, in 96-deep-wells. In each well, 250 μL of a solution of 700 μM of hemin in 25 mM NaOH were added to 250 μL of a suspension of 1 mM 1-monooleoylglycerol in 90 mM sodium acetate at pH 5. Drugs were added from DMSO stock solutions (5 μL). Microplates were incubated for 24 h at 37 °C. Controls contained an equal amount of DMSO. Following incubation, the samples were centrifuged at 4000 tr/min at 4 °C for 30 min. The pellet of β-hematin was washed with 10 mM sodium phosphate, pH 7.4, containing 2.5% SDS, and was vortexed for 10 min at 20 °C before repelleting until the supernatant was colorless (5 times). Dissolution of β-hematin was achieved by addition of 450 μL of 10 mM sodium phosphate, pH 7.4, containing 2.5% SDS and 25 μL of NaOH 1 M. Concentration of haem was calculated from absorbance at 405 nm.

5.2.4. In vivo drug assays on P. berghei

Antimalarial activity was determined in mice infected with *P. berghei* (ANKA 65 strain). Four-week-old female Swiss mice (CD-1, 20 g–25 g) were intraperitoneally infected with about 10^7 parasitized erythrocytes, collected from the blood of an acutely infected donor animal. At the same time, the animals (three per group) were orally treated with the test compound at 40 mg/kg (drug formulation in 100% DMSO). In order not to interfere with the IP infection, this first treatment dose is given orally. The treatment was continued during the four following days by the intraperitoneal route because the absorption is more extensive with less first-pass metabolism, if any, again maximizing the treatment to show activity. Untreated control animals generally die between 7 and 10 days following infection. Drug activity was evaluated by the reduction of parasites on day 4 and the prolongation of the mean survival time compared to that of untreated controls. Three infected DMSO-dosed mice were used as controls.

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