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Synthesis, antimalarial activity, and cellular toxicity of new arylpyrrolylaminoquinolines

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ABSTRACT

A set of nine new arylpyrrolyl derivatives of 7-chloro-4-aminoquinoline, characterized by different substituents on the phenyl ring or different distance between the pyrrolic nitrogen and the 4-aminoquinoline, has been synthesized and tested for their activity against D-10 (CQ-S) and W-2 (CQ-R) strains of *Plasmodium falciparum.* All compounds exhibited activity against the CQ-S strain in the low nM range, comparable to that of chloroquine. Some of them were also highly active against the CQ-R strain and not toxic against normal cells. The antimalarial activity of this new class of compounds seems to be related to the inhibition of heme detoxification process of parasites, as in the case of chloroquine.

1. Introduction

Malaria is estimated to kill more than 1 million people annually and most of these are children under the age of five.[1]

Presently, the most promising and successful strategy in fighting malaria is artemisinin-based combination therapy (ACT), including artemisinin or one of its derivatives, which are all rapidly eliminated, combined with a more slowly eliminated drug such as lumefantrine, amodiaquine, mefloquine, or sulfadoxine-pyrimetamine.[2] However the recent reports of ACT failure in South East Asia and the potential emergence of artemisinin resistance[3] indicate that the search for new drugs or new combinations is still highly necessary.

In order to develop new classes of antimalarial agents, we recently explored the possibility of replacing the phenolic ring of amodiaquine and tebuquine with other kinds of aromatic rings such as a pyrrole nucleus, still linked to the quinoline moiety through the usual NH. We showed that this new class of compounds (Fig. 1) is associated with a good antimalarial activity against both chloroquine sensitive (CQ-S) and chloroquine-resistant (CQ-R) strains of *Plasmodium falciparum*. In particular, we noted that the activity increased with the increasing of lipophilicity as shown by 2-methyl-5-(4-chlorophenyl) and 2-methyl-5-phenyl derivatives (**3a, 3b**, and **2a–2f**, respectively) which were more active than the corresponding 2,5-dimethyl derivatives (**1a–1f**). We observed also that small basic heads such as the diethylamino and pyrrolidino moieties were more profitable when a phenyl substituent was present on the pyrrole ring compared to bulkier ones, such as the quinolizidinylmethylamino group which was advantageous within the 2,5-dimethylpyrrole subset of compounds.[4]

Starting from these first evidences, we explored more deeply the electronic and lipophilic requirements for the antimalarial activity of this class of compounds. In particular, the role of the substituent in the *para* position of the phenyl ring, as well as the role of the distance between the pyrrolic nitrogen and the 4-aminoquinoline was investigated. A set of new 7-chloro-4-[*N*-[2-methyl-[5-(4-R-phenyl)]-1*H*-pyrrol-1-yl]amino]quinolines (**4–7**) and 7-chloro-4-[*N*-[3-[5-(4-chlorophenyl)-2-methyl-1*H*-pyrrol-1-yl]propyl]amino]quinolines (**8a**, **8b**) bearing a diethylaminomethyl or a pyrrolidinomethyl moiety as basic head was synthesized (Fig. 2).

Experiments were conducted to evaluate the β hematin inhibitory activity and the lipophilicity of these compounds and thus to get some insights on their mechanism of action.

2. Chemistry

Compounds **4–6** and **8** were synthesised through the condensation of the appropriate α-(4-substituted-phenacyl)-acetoacetate (**11**–**15**) with 7-chloro-4-hydrazinoquinoline **9** (Scheme 1) and the obtained pyrrole derivatives (**16–18** and **20**) were converted to amides (**21a**,**b–24a**,**b**) by the action of relevant amines in presence of trimethylaluminium in toluene[5,6] heating at 150 °C in a microwaves synthesiser system. The use of microwaves allowed us to shorten heating time from the 24 h previously required[4] to only 35 min and to improve the yield, especially of compound **22b** (49% vs <10% with the traditional method).

Amides were then reduced to the expected aminocompounds **4–6**, **8** by the means of diphenylsilane in the presence of tris(tri-

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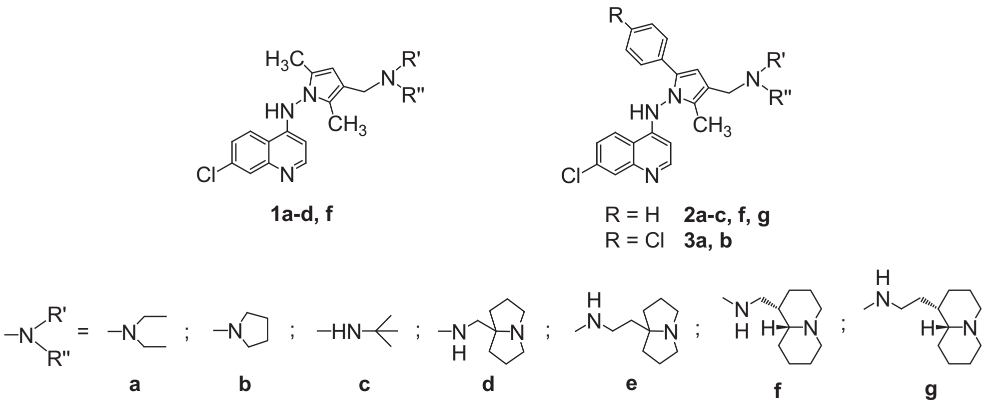


Figure 1. Structures of 4-[(pyrrol-1-yl)amino]quinoline derivatives with antimalarial activity.

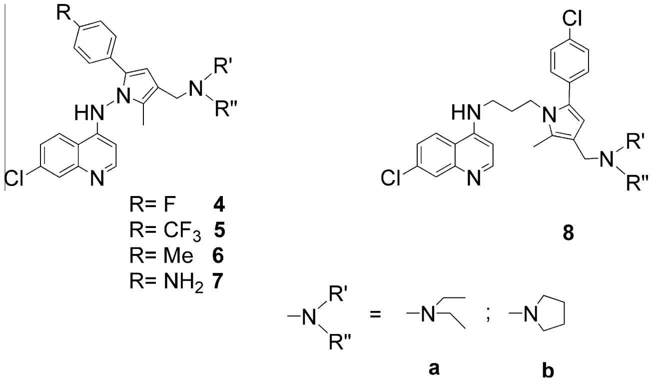


Figure 2. Structures of the investigated compounds.

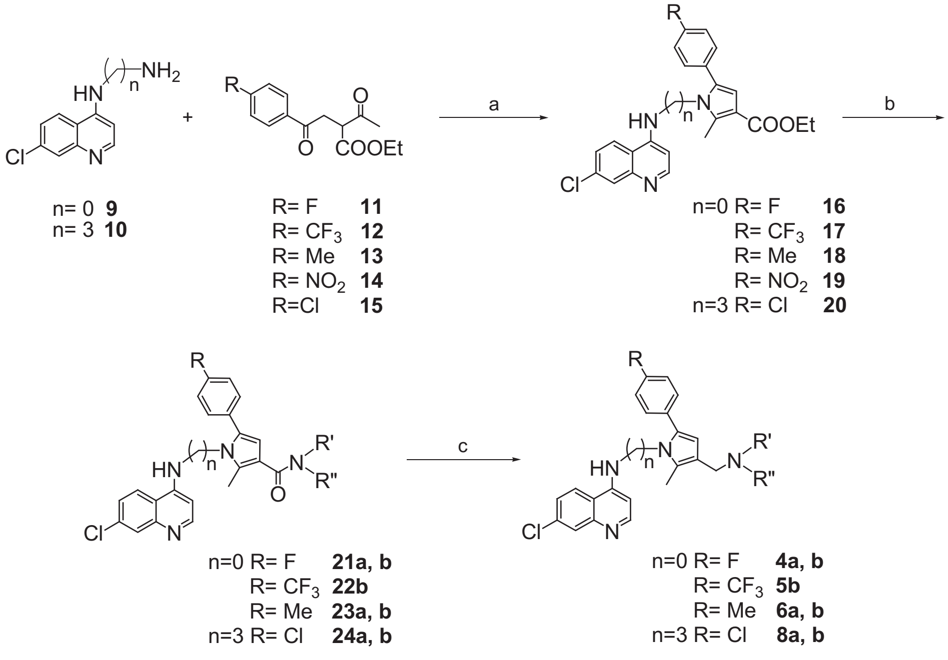
phenylphosphine)rhodium(I)carbonylhydride,[7,8] thus preventing the dehalogenation of the phenyl ring. Compounds **26a** and **26b** could not be obtained through the above described method, probably due to the electronic effect of nitro group. Thus a different synthetic pathway was set up (Scheme 2): compound **19** was hydrolyzed[9,10] to the corresponding carboxylic acid **25** which was coupled with the pertinent amine in presence of DCC and HOBt.[11] Nitro- and amidogroups were than reduced with iron in acetic acid and with $LiAlH_4$ in THF,[12,13] respectively, in order to obtain compounds **7a** and **7b**.

Starting 4-[*N*-(3-aminopropyl)amino]-7-chloroquinoline **10** was obtained as described by Solomon et al.[14] with small modifications.

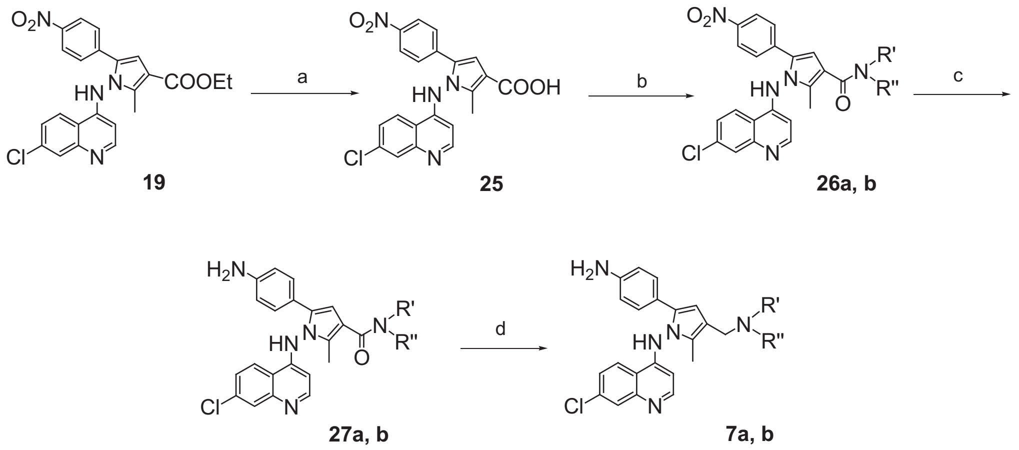
Finally, the required diketoesters of Scheme 1 were prepared by reacting 4-substitutedphenacyl bromide with sodium ethyl acetoacetate.[15–17]

3. Results and discussion

The compounds of Figure 2 were tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. Their antimalarial activity was quantified as inhibition of parasite growth[18] and was compared to the cytotoxicity[19] on mammalian cell lines both of human and murine origin (Table 1). All tested compounds exhib-



Scheme 1. Reagents and conditions: (a) AcOH, reflux; (b) $AlMe_3$, HNR′R″, PhMe, m.w.; (c) Ph_2SiH_2, $(PPh_3)_3(CO)HRh$, THF.



Scheme 2. Reagents and conditions: (a) LiOH, EtOH, H_2O, reflux; (b) HNR′R″, DCC, HOBt, DMF, m.w., 55 °C; (c) Fe, AcOH, EtOH, H_2O, 80 °C; (d) $LiAlH_4$, THF, reflux.

Table 1
In vitro antimalarial activities, β-hematin inhibitory activity (BHIA assay) and cellular cytotoxicity of the compounds under study

Compound	D-10 (CQ-S) IC_{50}[a] (nM)	Ratio IC_{50} CQ/compd[b]	W-2 (CQ-R) IC_{50}[a] (nM)	Ratio IC_{50} CQ/compd[b]	Ratio IC_{50} CQ-R/CQ-S[c]	HMEC-1 CC_{50}[d] (nM)	K562 CC_{50}[d] (nM)	WEHI-13 CC_{50}[d] (nM)	HDF CC_{50}[d] (nM)	BHIA IC_{50}[a,f] (molar equivalents to hemin)
4a	61.6 ± 7.2	0.5	79.8 ± 18.0	5.0	1.3	18606 ± 1252	2357 ± 641	3021 ± 595	n.t.	n.t.
4b	32.6 ± 2.7	0.8	78.1 ± 28.0	5.7	2.4	10070 ± 1356	1655 ± 368	2713 ± 414	n.t.	n.t.
5b	58.0 ± 13.0	0.5	59.0 ± 16.6	6.7	1.0	4701 ± 2928	1217 ± 454	2680 ± 206	n.t.	n.t.
6a	32.7 ± 2.8	0.6	55.4 ± 16.8	6.4	1.7	7051[e]	n.t.	3653 ± 700	10265[e]	n.t.
6b	30.7 ± 2.0	0.6	65.8 ± 12.7	5.4	2.1	7151[e]	n.t.	4480 ± 988	13185[e]	0.45 ± 0.07
7a	53.4 ± 20.0	0.5	313.4 ± 66.0	0.9	5.8	n.t.	n.t.	n.t.	n.t.	n.t.
7b	43.1 ± 11.9	0.5	455.1 ± 57.2	0.5	10.6	n.t.	n.t.	n.t.	n.t.	n.t.
8a	26.0 ± 6.9	0.7	179.6 ± 45.9	2.3	6.9	n.t.	n.t.	n.t.	n.t.	n.t.
8b	22.7 ± 5.1	0.8	243.4 ± 52.4	1.7	10.7	n.t.	n.t.	n.t.	n.t.	0.82 ± 0.09
2a[g]	42.7 ± 8.9[g]	0.7	59.2 ± 27.4[g]	9.9	1.4	16694 ± 5919[g]	3616 ± 1268[g]	4380 ± 2105[g]	n.t.	n.t.
2b[g]	42.9 ± 13.0[g]	0.7	139.5 ± 56.0[g]	4.2	3.3	12874 ± 2824[g]	2920 ± 866[g]	3878 ± 638[g]	n.t.	0.54 ± 0.16
3a[g]	26.1 ± 8.3[g]	0.9	42.5 ± 19.1[g]	18.5	1.6	5812 ±1511[g]	n.t.	1446 ± 670[g]	n.t.	n.t.
3b[g]	30.7 ± 4.3[g]	0.8	49.6 ± 5.2[g]	15.8	1.6	7176 ± 1383[g]	n.t.	2060±125[g]	n.t.	0.36 ± 0.07
CQ	24.8 ± 4.1	-	440.7 ± 171.4	-	17.8	>32000	>25000	>38000	>38000	1.27 ± 0.13

n.t., not tested.
[a] The results are expressed as IC_{50} ± SD of at least three different experiments each performed in duplicate or triplicate.
[b] Mean of ratios between the IC_{50} of chloroquine and that of each compound against D-10 or W-2 strains of *P. falciparum* calculated for each single experiment.
[c] Ratios between the IC_{50} values of each compound against the two strains of *P. falciparum.*
[d] The cytotoxic activity was assayed in vitro on different cell line using the MTT assay.
[e] Mean of two experiments each performed in duplicate.
[f] The IC_{50} indicate the moles of compounds, relative to hemin, required to inhibit beta-hematin formation by 50%.
[g] From Ref. 4.

ited high activity on the CQ-S (D-10) strain, with IC_{50} ranging from 22.7 to 61.6 nM. The IC_{50} of CQ was 24.7 nM (range 19.5–29.6 nM), thus the tested compounds were from 0.5- to 0.8-fold as active as CQ (Table 1). Five of the new compounds exhibited also a strong activity against the CQ-R (W-2) strain, resulting from 5 to 6.7 times more active than CQ, with IC_{50} values as low as 55.4–79.8 nM (compounds **4a, 4b, 5b, 6a, and 6b**) compared to 440.7 nM of CQ (range 252–784 nM). Compounds **8a, 8b, 7a**, and **7b**, although in some case more active than CQ on CQ-R strain, showed evident cross resistance. In fact, the resistance factor, calculated as the ratios between the IC_{50} of each compound against the two strains of *P. falciparum* was significantly higher (range 5.8–10.7) for compounds **8a, 8b, 7a**, and **7b**, compared to**4a, 4b, 5b, 6a, 6b**, for which the resistance factor ranged from 1.0 to 2.4, resulting about 7–17 times lower than that of CQ (17.8). This suggests that the latter compounds were not (or only poorly) affected by the resistance mechanisms.

Table 1 also shows the data related to the previously synthesized compounds **2a**, **2b**, **3a**, and **3b**[4] for a easier comparison.

The most active compounds exhibit low or moderate toxicity against three different human or mouse cell lines with a therapeutic index, calculated on W-2 strain, ranging between 21 and 233.

In addition, the ability of some representative compounds (**2b**, **3b**, **6b**, and **8b**; Table 1) to inhibit β-hematin formation was evaluated using the BHIA (β-hematin inhibitory activity) assay.[20] Compared to CQ, all of them showed a stronger ability to interfere with the process of heme crystallization, with IC_{50} significantly lower than that of CQ.

From these results it is evident that the hydrophilic amino group of compounds **7a** and **7b** is not a suitable substituent for the activity on CQ-R strains of *P. falciparum.* With the aim to better understand the influence of lipophilicity (of the substituent in *para* on the phenyl ring), we measured pK_a and log *P* of the compounds **4b**, **5b**, **6b**, and **7b**, as well as those of the previously synthesized compounds **2b** and **3b** (with R = H and Cl, respectively) all bearing a pyrrolidine moiety as basic head. The results are collected in Table 2. The determined pK_{a1}, pK_{a2}, log *P* and log *D* values obtained for CQ are in good agreement with the data previously reported.[21]

Table 2

Physicochemical characteristics of compounds bearing a pyrrolidine moiety as basic head.[a]

Compound	pK_{a1}	pK_{a2}	pK_{a3}	Log P	Log $D_{7.4}$		Log$D_{5.2}$		LAR	
					Obs.	Calc.[b]	Calc.[b]	VAR $_{\text{pH 5.2}}$[c]	Obs.[d]	Calc.[e]
4b	8.84 ± 0.02	6.20 ± 0.02	–	4.86 ± 0.02	3.45	3.37	0.18	1548.8	2818.4	2344.2
5b	7.89 ± 0.02	6.05 ± 0.02	–	5.28 ± 0.18	4.72	4.65	1.69	912.0	52480.8	44668.4
6b	8.68 ± 0.03	6.28 ± 0.03	–	5.19 ± 0.06	3.91	3.86	0.59	1862.1	8128.3	7244.4
7b	9.78 ± 0.03	6.47 ± 0.03	3.79 ± 0.03	4.38 ± 0.02	2.03	1.95	−1.50	16982.4	107.1	89.1
8b	8.46 ± 0.01[f]	[f]	–	4.38 ± 0.04	2.35	2.22	−2.21	24547.1	223.9	166.0
2b	8.38 ± 0.02	6.26 ± 0.03	–	4.60 ± 0.01	3.28	3.55	0.32	1698.2	1905.5	3548.1
3b	8.52 ± 0.03	6.20 ± 0.03	–	5.17 ± 0.06	4.06	3.99	0.81	1513.6	11481.5	9772.4
CQ	10.20 ± 0.03	8.41 ± 0.03	–	4.73 ± 0.01	0.90	0.88	−3.49	22908.7	7.9	7.4

[a] pK_a, log P, and log D values were calculated as described in Section 5. The experiments were conducted at 25 °C. Obs, observed; Calc, calculated; log $D_{7.4}$ and log $D_{5.2}$, log D values at pHs 7.4 and 5.2, respectively.

[b] Calculated from the following equation: $\log D = \log P - \log[1 + 10^{(\mathrm{p}K\mathrm{a1}-\mathrm{pH})} + 10^{(\mathrm{p}K\mathrm{a1}+\mathrm{p}K\mathrm{a2}-2\mathrm{pH})}]$.

[c] VAR, antilog (calculated log $D_{7.4}$ − calculated log $D_{5.2}$).

[d] Antilog for observed log $D_{7.4}$.

[e] Antilog for calculated log $D_{7.4}$.

[f] The pK_a values of the two basic moieties are practically superimposed, since the presence of the trimethylene spacer restored the basicity of the 4-aminoquinoline moiety to the level of CQ.

As shown, log P values of the novel compounds and CQ are very close to each other (4.38–5.28). On the contrary, the distribution coefficients at pH 7.4 are quite different with log D values spread from 4.72 (**5b**, R = CF_3) to 2.03 (**7b**, R = NH_2), while log D of CQ was still lower (0.90). The lower basicity of compounds **4b**, **5b**, **6b** (pK_{a1} = 8.84, 7.89, and 8.68, respectively) compared to CQ (pK_{a1} = 10.20), implies that at physiological pH they are less protonated. These features might improve the cellular permeation of these compounds at physiological pH. At pH 5.2 (which is supposed to reflect the pH of the digestive vacuole),[22] compounds **2b-6b** display a stronger lipophilic character than compounds **7b** and **8b** and even more than CQ (log $D_{5.2}$ = −3.49) (Table 2). This means that, whereas CQ concentrates predominantly in the aqueous regions, compounds **2b–6b** should concentrate in the lipid regions or at the interface between lipids and water. This may facilitate their interaction with heme molecules during the crystallization process to hemozoin which is reported to occur in vitro at the lipid–water interface or in vivo within neutral lipid nanosphere.[23,24] This is partly confirmed by the strong inhibition of β-hematin formation shown by compounds **6b**, **8b** and **2b**, **3b** (Table 1).

In agreement, LAR (lipid accumulation ratio) values are significantly higher for compounds **4b, 5b, 6b**, and **3b** compared to compounds **7b, 8b** and CQ. This finding may indicate the ability of the former compounds to block drug efflux by hydrophobic interaction with PfCRT (*Plasmodium falciparum* Chloroquine Resistance Transporter) and explain the lower RI (resistance index) and the higher efficacy toward CQ-R strains of *P. falciparum.*[25]

It has been reported that a high VAR (vacuolar accumulation ratio) value in addition to the ability of inhibiting in vitro the formation of β-hematin is important for activity against CQ-S parasites,[25] however in our set of compounds, VAR values do not seem well correlated to the strong activity against the CQ-S strain of *P. falciparum*, suggesting that the interaction of hematin with uncharged drug species may be more important than previously suspected, as also observed by O'Neill et al.[26]

As shown in Figure 3, among the homogeneous set of compounds bearing a pyrrolidine ring as basic head, the most lipophilic compounds **5b**, **3b**, and **6b** (R = CF_3 and Cl and CH_3, respectively), were also the most active against CQ-R strain of *P. falciparum* whereas the least lipophilic 4-amino substituted **7b** was the least active. It is worth noting that conforming to expectation,[27] the 4-fluorosubstituted derivative **4b** exhibited higher log P and log $D_{7.4}$ values in respect to the unsubstituted compound **2b**, and the activity of **4b** was higher than that of **2b**, however the log D at pH 5.2 was lower. A multivariate analysis (Minitab 14 software) of the results concerning compounds **2b–7b** suggests a good correlation (R^2 = 0.975) between activity and lipophilicity. However, the synthesis and the evaluation of additional compounds with log $D_{5.2}$ in the region below 0.0 would be needed to provide a more sound relation.

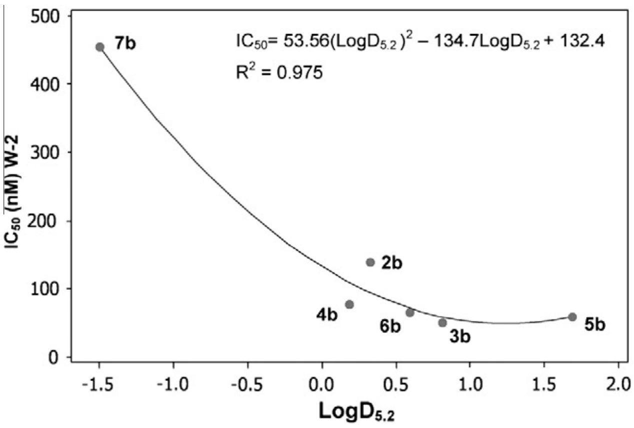


Figure 3. Relationship between log $D_{5.2}$ and IC_{50} on W-2 strain of *P. falciparum* of the compounds **2b**-**7b**.

Anyway the present results are in agreement with the data previously reported for other classes of aminoquinoline derivatives.[25,26,28,29]

4. Conclusions

A set of new 7-chloro-4-[*N*-[2-methyl-[5-(4-R-phenyl)]pyrrol-1-yl]amino]quinolines (**4a,4b–7a,7b**) and 7-chloro-4-[*N*-[3-[5-(4-chlorophenyl)-2-methylpyrrol-1-yl]propyl]amino]quinolines (**8a**, **8b**) bearing a diethylaminomethyl or a pyrrolidinomethyl moiety as basic head was synthesized, in order to explore the best electronic and lipophilic requirements for the antimalarial activity of this class of compounds. In particular, the role of the substituent in the *para* position of the phenyl ring, as well as the role of the distance between the pyrrolic nitrogen and the 4-aminoquinoline was investigated. Indeed, the most lipophilic compounds **5b**, **3b**, and **6b** were also the most active on W2 (CQ-R) strain of *P. falciparum*, while the presence of a hydrophilic substituent on the phenyl ring, although maintains the activity against CQ-S strain, was detrimental for the activity on CQ-R strain. The increase of the distance between the pyrrolic nitrogen and the 4-aminoquinoline was not profitable for the resistance index.

Similarly to other aminoquinoline derivatives, this new class of compounds seems able to interfere with the heme detoxification process of parasites.

5. Experimental

5.1. General

All commercially available solvents and reagents were used without further purification, unless otherwise stated. CC = flash column chromatography. Mps: Büchi apparatus, uncorrected. ^{1}H NMR spectra: Varian Mercury 300VX spectrometer; $CDCl_3$ or DMSO-d_6; δ in ppm, J in Hertz. High-resolution mass spectra (HRMS) on a APEX II ICR-FTMS Bruker Daltonics mass spectrometer in positive electro spray ionization (ESI). Elemental analyses were performed on a *Carlo Erba EA-1110* CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmaceutical Sciences of Genoa University. Where indicated, reactions were performed with a Biotage Iniziator Microwave Synthesis System.

5.2. Ethyl 2-acetyl-4-oxo-4-[(4-substituted)phenyl]butanoate (11–15): general method

According to literature,[15–17]ethyl acetoacetate (1.56 g, 12 mmol) was added dropwise to a stirred suspension of NaH 60% p/p (10 mmol) in 15 ml of anhydrous diethyl ether at rt under N_2. After 10 min a solution of 4-substituted phenacylbromide (10 mmol) in 26 ml of anhydrous diethyl ether was added dropwise, and the resulting mixture was heated at reflux for 2 h. After cooling, the NaBr was filtered, washed twice with diethyl ether, and the joined organic phases were evaporated to dryness and the crude product purified by CC (silica gel; elution conditions as indicated for each compound).

5.2.1. Ethyl 2-acetyl-4-(4-fluorophenyl)-4-oxobutanoate (11)[17]

CC (cyclohexane/AcOEt; 80:20); solid crystallized with diethyl ether and rinsed with petroleum ether. Yield: 59%. Mp 56.5–58 °C. ^{1}H NMR ($CDCl_3$): δ 8.03–7.98 (m, 2H); 7.16–7.10 (m, 2H); 4.26–4.19 (m, 3H); 3.68 (dd, J = 8.25, 18.43 Hz, 1H); 3.47 (dd, J = 5.78, 18.43 Hz, 1H); 2,44 (s, 3H); 1.29 (t, J = 7.15 Hz, 3H).

5.2.2. Ethyl 2-acetyl-4-[4-(trifluoromethyl)phenyl]-4-oxobutanoate (12)

CC (cyclohexane/AcOEt; 93:7); solid crystallized with diethyl ether and rinsed with petroleum ether. Yield: 78%. Mp 63.8–66.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.08 (d, J = 7.98 Hz, 2H); 7.73 (d, J = 8.26 Hz, 2H); 4.27–4.20 (m, 3H); 3.72 (dd, J = 8.25, 18.43 Hz, 1H); 3,50 (dd, J = 5.77, 18.47 Hz, 1H); 2.45 (s, 3H); 1.30 (t, J = 7.15 Hz, 3H).

5.2.3. Ethyl 2-acetyl-4-*p*-tolyl-4-oxobutanoate (13)

CC (cyclohexane/AcOEt; 70:30); solid rinsed with petroleum ether/diethyl ether (90:10). Yield: 70%. Mp 39.5–42 °C. ^{1}H NMR ($CDCl_3$): δ 7.87 (d, J = 7.97 Hz, 2H); 7.25 (d, J = 7.7 Hz, 2H); 4.25–4.18 (m, 3H); 3.69 (dd, J = 8.26, 18.43 Hz, 1H); 3.49 (dd, J = 5.77, 18.43 Hz, 1H); 2.43 (s, 3H); 2.41 (s, 3H); 1.29 (t, J = 7.15 Hz, 3H).

5.2.4. Ethyl 2-acetyl-4-(4-nitrophenyl)-4-oxobutanoate (14)

CC (cyclohexane/AcOEt; 85:15); solid rinsed with a mixture of petroleum ether/diethyl ether (60:40). Yield: 61%. Mp 47.8–48.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.32 (d, J = 9.08 Hz, 2H); 8.13 (d, J = 9.08 Hz, 2H); 4.28–4.20 (m, 3H); 3.73 (dd, J = 18.70, 8.25 Hz, 1H); 3.49 (dd, J = 18.43, 5.50 Hz, 1H); 2.44 (s, 3H); 1.30 (t, J = 7.16, 3H).

5.2.5. Ethyl 2-acetyl-4-(4-chlorophenyl)-4-oxobutanoate (15)[16]

CC (cyclohexane/AcOEt; 60:40); solid rinsed with petroleum ether/diethyl ether (80:20). Yield: 81%. Mp 64.8–66.6 °C. ^{1}H NMR ($CDCl_3$): δ 7.92 (dd, J = 1.93, 6.88 Hz, 2H); 7.43 (dd, J = 1.93, 6.88 Hz, 2H); 4.25–4.18 (m, 3H); 3.67 (dd, J = 18.40, 8.25 Hz, 1H); 3.43 (dd, J = 18.40, 8.25 Hz, 1H); 2.43 (s, 3H); 1.30 (t, J = 6.82 Hz, 3H).

5.3. *N*-(3-Aminopropyl)-4-amino-7-chloroquinoline (10)[14]

In a round bottom flask propane-1,3-diamine (10 ml, 120 mmol) and 4,7-dichloroquinoline (2 g, 10.1 mmol) were added. The mixture was heated to reflux under N_2 atmosphere for 3.5 h and then cooled in ice-bath, diluted with water and added with 2 M NaOH until complete precipitation. Precipitate was filtered, washed with water, diethyl ether, and dried over anhydrous Na_2SO_4. The crude product was chromatographed on a column of silica gel (CH_2Cl_2/MeOH/concd NH_3; from 80:18:2 to 75:22.5:2.5). The resulted solid was washed with diethyl ether. Yield: 78%. Mp 80–83 °C (lit.[14] mp 96–98 °C). ^{1}H NMR (DMSO-d_6): δ 8.30 (d, J = 5.50 Hz, 1H); 8.12 (d, J = 9.07 Hz, 1H); 7.74 (d, J = 2.20 Hz, 1H); 7.50 (br s, 1H); 7.38 (dd, J = 2.20, 9.08 Hz, 1H); 6.45 (d, J = 5.50 Hz, 1H); 3.50–3.00 (m, 4H); 2.57 (t, J = 6.88 Hz, 2H); 1.74–1.64 (m, 2H).

5.4. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-[(4-substituted)phenyl]-1*H*-pyrrole-3-carboxylate (16–19) and ethyl 1-[3-((7-chloroquinolin-4-yl)amino)propyl]-2-methyl-5-(4-chlorophenyl)-1*H*-pyrrole-3-carboxylate (20): general method

The suitable diketoester (**11–15**; 6.2 mmol) was added to a stirred solution of 7-chloro-4-hydrazinoquinoline (**9**) or *N*-(3-aminopropyl)-4-amino-7-chloroquinoline (**10**; 6.2 mmol) in 10 ml of glacial acetic acid. The mixture was refluxed under N_2 for 2 h to give a violet solution, which, after cooling, was alkalized with cold 2 N NaOH and extracted three times with CH_2Cl_2. The joined organic layers were dried (Na_2SO_4); the solvent was removed and the crude solid was purified by CC (silica gel; different ratio of ethyl acetate/cyclohexane or CH_2Cl_2/MeOH as indicated for each compound).

5.4.1. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (16)

CC (AcOEt/cyclohexane; 20:80); solid crystallized with diethyl ether and rinsed with petroleum ether/diethyl ether (70:30). Yield 76%. Mp 186.4–188.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.17–8.14 (m, 2H); 7.87 (s, 1H); 7.44 (dd, J = 1.93, 9.08 Hz, 1H); 7.39–7.34 (m, 2H); 6.90–6.85 (m, 2H); 6.71 (s, 1H); 5.83 (d, J = 5.77 Hz, 1H); 4.29 (q, J = 7.15 Hz, 2H); 2.40 (s, 3H); 1.36 (t, J = 7.15 Hz, 3H).

5.4.2. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-[4-(trifluoromethyl)phenyl]-1*H*-pyrrole-3-carboxylate (17)

CC (AcOEt/cyclohexane; 15:85); solid rinsed with petroleum ether/diethyl ether (50:50). Yield 69%. Mp 191–193 °C. ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 8.81 Hz, 1H); 7.94 (br s, 1H); 7.80 (s, 1H); 7.61 (d, J = 7.97 Hz, 2H); 7.47–7.40 (m, 3H); 6.86 (s, 1H); 5.73 (d, J = 6.06 Hz, 1H); 4.30 (q, J = 6.88 Hz, 2H); 2.41 (s, 3H); 1.37 (t, J = 6.88 Hz, 3H).

5.4.3. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-*p*-tolyl-1*H*-pyrrole-3-carboxylate (18)

CC (AcOEt/cyclohexane; 20:80); resulting solid rinsed with petroleum ether/diethyl ether (50:50). Yield 78%. Mp 180–181 °C.

^{1}H NMR ($CDCl_3$): δ 8.22–8.13 (m, 2H); 7.97 (s, 1H); 7.46 (dd, J = 2.2, 9.07 Hz, 1H); 7.27–7.25 (m, 2H); 6.98 (d, J = 7.97 Hz, 2H); 6.68 (s, 1H); 5.86 (d, J = 5.77 Hz, 1H); 4.29 (d, J = 6.87 Hz, 2H); 2.41 (s, 3H); 2.22 (s, 3H); 1.36 (t, J = 7.1 Hz, 3H).

5.4.4. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (19)

CC (AcOEt/cyclohexane; 30:70); solid rinsed with petroleum ether/diethyl ether (50:50). Yield 73%. Mp 242.3–244.5 °C. ^{1}H NMR (CD_3OD): δ 8.50–8.20 (br s, 2H); 8.06 (d, J = 8.25, 2H); 7.81 (d, J = 7.42 Hz, 2H); 7.65–7.30 (m, 2H); 7.07 (s, 1H); 4.33 (q, J = 6.60 Hz, 2H); 2.41 (s, 3H); 1.37 (t, J = 7.15 Hz, 3H).

5.4.5. Ethyl 1-[3-((7-chloroquinolin-4-yl)amino)propyl]-2-methyl-5-(4-chlorophenyl)-1*H*-pyrrole-3-carboxylate (20)

CC (CH_2Cl_2/MeOH; 98:2); solid crystallized with a mixture of CH_2Cl_2/diethyl ether and rinsed with diethyl ether. Yield 75%. Mp 148.5–151.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.40 (d, J = 5.50 Hz, 1H); 7.94 (s, 1H); 7.34–7.20 (m, 7H); 6,55 (s, 1H); 6.16 (d, J = 5.50 Hz, 1H); 4.27 (q, J = 6.88 Hz, 2H); 4.15 (t, J = 6.60 Hz, 2H); 3.14–3.08 (m, 2H); 2.62 (s, 3H); 1.98–1.90 (m, 2H); 1.34 (t, J = 6.88 Hz, 3H).

5.5. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-[(4-substituted)phenyl]-1*H*-pyrrole-3-(*N*-substituted)carboxamide (21a,b–23a,b, 24a,b): general method

In a microwaves vial, 2 M $Al(CH_3)_3$ in toluene (0.725 ml, 1.5 mmol) was added dropwise to an ice-cooled stirred solution of the appropriate amine (1.5 mmol) in 3 ml of anhydrous toluene. The resulting mixture was stirred under N_2 at rt for 30 min, then the relevant ester derivative (**13–15, 17**; 1 mmol) was added. The solution was heated with microwaves (150 °C, 35 min), cooled and then treated with 2 N NaOH solution. The precipitate was filtered and washed four times with AcOEt, the organic phase was separated, dried (Na_2SO_4) and evaporated. The solid was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.5.1. 1-[(7-Chloroquinolin-4-yl)amino]-5-(4-fluorophenyl)-2-methyl-1*H*-pyrrole-3-(*N,N*-diethyl)carboxamide (21a)

CC (CH_2Cl_2/MeOH; 98.5:1.5); solid rinsed with diethyl ether. Yield: 62%. Mp 222–223 °C. ^{1}H NMR (DMSO-d_6): δ 10.69 (s, 1H); 8.44 (d, J = 4.67 Hz, 1H); 8.27 (d, J = 9.36 Hz, 1H); 7.91 (s, 1H); 7.64–7.35 (m, 3H); 7.12–6.98 (m, 2H); 6.49 (s, 1H); 5.69 (d, J = 5.23 Hz, 1H); 3.40 (q, J = 6.60 Hz, 4H); 2.20 (s, 3H); 1.13 (q, J = 6.60 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{25}H_{25}ClFN_4O$ $[M+H]^+$: 451.16954; found: 451.16926.

5.5.2. [1-((7-Chloroquinolin-4-yl)amino)-5-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (21b)

During the reaction, a suspension was formed and a first portion of compound **21b** was filtered, washed with diethyl ether and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 97:3); solid rinsed with diethyl ether. Yield: 45%. Mp 310–313 °C. ^{1}H NMR (DMSO-d_6): δ 10.69 (s, 1H); 8.43 (d, J = 5.23 Hz, 1H); 8.27 (d, J = 8.81 Hz, 1H); 7.91 (s, 1H); 7.64–7.35 (m, 3H); 7.11–6.98 (m, 2H); 6.70 (s, 1H); 5.70 (d, J = 4.96 Hz, 1H); 3.65 (br s, 2H); 3.44 (br s, 2H); 2.18 (s, 3H); 1.85 (br s, 4H). HRMS (ESI) *m/z* calcd for $C_{25}H_{23}ClFN_4O$ $[M+H]^+$: 449.15389; found: 449.15353.

5.5.3. [1-((7-Chloroquinolin-4-yl)amino)-5-(4-(trifluoromethyl)-phenyl)-2-methyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (22b)

During the reaction, a suspension was formed and a first portion of compound **22b** was filtered, washed with diethyl ether and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 97.5:2.5); solid rinsed with diethyl ether. Yield: 49%. Mp 318–320 °C (dec). ^{1}H NMR (CF_3COOD): δ 8.50 (d, J = 6.07 Hz, 1H); 8.38 (d, J = 9.36 Hz, 1H); 8.02 (s, 1H); 7.85 (d, J = 9.62 Hz, 1H); 7.56 (s, 4H); 6.80 (s, 1H); 6.27 (d, J = 6.05 Hz, 1H); 3.99 (br s, 4H); 2.41 (s, 3H); 2.23 (br s, 4H). HRMS (ESI) *m/z* calcd for $C_{26}H_{23}ClF_3N_4O$ $[M+H]^+$: 521.13264; found: 521.13199.

5.5.4. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-*p*-tolyl-1*H*-pyrrole-3-(*N,N*-diethyl)carboxamide (23a)

CC (CH_2Cl_2/MeOH; 90:10); solid rinsed with petroleum ether/diethyl ether (80:20). Yield: 82%. Mp 255.1–256 °C. ^{1}H NMR (DMSO-d_6): δ = 10.65 (br s, 1H); 8.58 (s, 1H); 8.30 (s, 1H); 7.88 (s, 1H); 7.60 (s, 1H); 7.42 (d, J = 7.70 Hz, 2H); 7.02 (d, J = 7.15 Hz, 2H); 6.41 (s, 1H); 5.65 (s, 1H); 3.42 (q, J = 6.85 Hz, 4H); 2.48 (s, 3H); 2.16 (s, 3H); 1.13 (t, J = 6.87 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{26}H_{28}ClN_4O$ $[M+H]^+$: 447.19462; found: 447.19450.

5.5.5. [1-((7-Chloroquinolin-4-yl)amino)-2-methyl-5-*p*-tolyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (23b)

During the reaction, a suspension was formed and a first portion of **23b** was filtered, washed with diethyl ether and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 97:3); solid rinsed with diethyl ether. Yield: 91%. Mp 190–191 °C. ^{1}H NMR (DMSO-d_6): δ 10.67 (s, 1H); 8.41 (d, J = 3.85 Hz, 1H); 8.29 (d, J = 8.53 Hz, 1H); 7.90 (s, 1H); 7.62 (d, J = 8.53 Hz, 1H); 7.41 (d, J = 7.98 Hz, 2H); 7.03 (d, J = 7.7 Hz, 2H); 6.6 (s, 1H); 5.66 (d, J = 4.4 Hz, 1H); 3.64 (br s, 2H); 3.44 (br s, 2H); 2.18–2.13 (m, 6H); 1.85 (s, 4H). HRMS (ESI) *m/z* calcd for $C_{26}H_{26}ClN_4O$ $[M+H]^+$: 445.17897; found: 445.17874.

5.5.6. 1-[3-((7-Chloroquinolin-4-yl)amino)propyl]-2-methyl-5-(4-chlorophenyl)-1*H*-pyrrole-3-(*N,N*-diethyl)carboxamide (24a)

CC (CH_2Cl_2/MeOH; 97:3); solid rinsed with diethyl ether. Yield: 85%. Mp 176.5–177.5 °C. ^{1}H NMR (DMSO-d_6): δ 8.33 (d, J = 5.23 Hz, 1H); 8.08 (d, J = 9.35 Hz, 1H); 7.76 (d, J = 1.92 Hz, 1H); 7.39 (dd, J = 1.93, 9.08 Hz, 1H); 7.25 (d, J = 8.53 Hz, 2H); 7.20–7.03 (m, 3H); 6.23 (d, J = 5.50 Hz, 1H); 6.08 (s, 1H); 4.10–4.00 (m, 2H); 3.32 (q, J = 6.90 Hz, 4H); 3.10–3.00 (m, 2H); 2.25 (s, 3H); 1.95–1.75 (m, 2H); 1.05 (t, J = 6.90 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{28}H_{31}Cl_2N_4O$ $[M+H]^+$: 509.18694; found: 509.18650.

5.5.7. [5-(4-chlorophenyl)-1-(3-((7-chloroquinolin-4-yl)amino)-propyl)-2-methyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (24b)

During the reaction, a suspension was formed and a first portion of **24b** was filtered, washed with diethyl ether and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 96:4); solid rinsed with diethyl ether. Yield: 78%. Mp 235.7–237 °C. ^{1}H NMR (DMSO-d_6): δ 8.33 (d, J = 5.22 Hz, 1H); 8.08 (d, J = 9.07 Hz, 1H); 7.42 (d, J = 2.20 Hz, 1H); 7.40 (dd, J = 2.20, 9.07 Hz, 1H); 7.25 (d, J = 8,53 Hz, 2H); 7.17 (br s, 1H); 7.09 (d, J = 8.52 Hz, 2H); 6.28 (s, 1H); 6.24 (d, J = 5.50 Hz, 1H); 4.04 (t, J = 7.15 Hz, 2H); 3.60–3.30 (m, 4H); 3.15–3.00 (m, 2H); 2.38 (s, 2H); 1.77 (s, 4H). HRMS (ESI) *m/z* calcd for $C_{28}H_{29}Cl_2N_4O$ $[M+H]^+$: 507.17129; found: 507.17078.

5.6. 7-Chloro-4-[*N*-[5-(4-substituted-phenyl)-3-[(*N*-substituted-amino)-methyl]-2-methyl-1*H*-pyrrol-1-yl]amino]quinoline (4a,b-6a,b-) and 7-chloro-4-[*N*-[3-[5-(4-chlorophenyl)-3-[(*N*-substituted-amino)methyl]-2-methyl-1*H*-pyrrol-1-yl]propyl]amine]quinoline (8a,b): general method

To a stirred suspension of corresponding amide (1 mmol) in 25 ml of anhydrous THF heated at reflux under N_2, tris(triphenylphosphine)rhodium(I) carbonyl hydride (45.94 mg, 0.05 mmol) and diphenylsilane (3.3 ml, 18 mmol) were added in five portions during 2 h and the mixture was further stirred for 3 h, until the amide was completely reduced. After cooling, THF was evaporated, and the residue was partitioned between CH_2Cl_2 and 0.5 N HCl. The acid phase was alkalized with a 2 N NaOH solution and extracted with CH_2Cl_2. The organic solution was dried (Na_2SO_4) and evaporated to yield the crude product that was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.6.1. 7-Chloro-4-[*N*-[2-methyl-3-[(diethylamino)methyl]-5-(4-fluorophenyl)-1*H*-pyrrol-1-yl]amino] quinoline (4a)

CC (CH_2Cl_2/MeOH; 93.5:6.5); solid rinsed with diethyl ether. Yield: 10%. Mp 225–229 °C (dec). ^{1}H NMR (DMSO-d_6): δ 10.62 (s, 1H); 8.39 (d, J = 4.95 Hz, 1H); 8.28 (d, J = 8.80 Hz, 1H); 7.89 (s, 1H); 7.59 (d, J = 8.80 Hz, 1H); 7.53–7.42 (m, 2H); 7.04 (t, J = 8.80 Hz, 2H); 6.36 (s, 1H); 5.59 (d, J = 4.95 Hz, 1H); 3.44 (s, 2H); 2.01 (s, 3H); 1.00 (t, J = 6.60 Hz, 6H); four protons are missing because covered by the DMSO signal. HRMS (ESI) *m*/*z* calcd for $C_{25}H_{27}ClFN_4$ [M+H]$^+$: 437.19028; found: 437.19192. Anal. Calcd for $C_{25}H_{26}ClFN_4$: C, 68.72; H, 6.00; N, 12.82. Found: C, 68.57; H, 6.31; N, 12.94.

5.6.2. 7-Chloro-4-[*N*-[2-methyl-3-(pyrrolidin-1-ylmethyl)-5-(4-fluorophenyl)-1*H*-pyrrol-1-yl]amino]quinoline (4b)

CC (CH_2Cl_2/MeOH; 94:6); solid rinsed with diethyl ether. Yield: 26%. Mp 170.5–173.5 °C. ^{1}H NMR (DMSO-d_6): δ 10.60 (s, 1H); 8.40 (d, J = 5.23 Hz, 1H); 8.28 (d, J = 9.07 Hz, 1H); 7.89 (s, 1H); 7.60 (d, J = 9.08 Hz, 1H); 7.53–7.48 (m, 2H); 7.04 (t, J = 9.08 Hz, 2H); 6.36 (s, 1H); 5.59 (d, J = 5.23 Hz, 1H); 3.46 (s, 2H); 2.01 (s, 3H); 1.68 (s, 4H); four protons are missing because covered by the DMSO signal. HRMS (ESI) *m*/*z* calcd for $C_{25}H_{25}ClFN_4$ [M+H]$^+$: 437.17463; found: 437.17569. Anal. Calcd for $C_{25}H_{24}ClFN_4$: C, 69.04; H, 5.56; N, 12.88. Found: C, 68.98; H, 5.85; N, 12.89.

5.6.3. 7-Chloro-4-[*N*-[2-methyl-3-(pyrrolidin-1-ylmethyl)-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrrol-1-yl]amino]quinoline (5b)

Solid obtained from reduction was not purified by CC but only rinsed with diethyl ether. Yield: 20%. Mp 187.5–190.2 °C. ^{1}H NMR (DMSO-d_6): δ 10.70 (s, 1H); 8.40 (br s, 1H); 8.32 (d, J = 6.60 Hz, 1H); 7.90 (s, 1H); 7.73 (d, J = 7.98 Hz, 2H); 7.60–7.53 (m, 3H); 6.58 (s, 1H); 5.60 (br s, 1H); 3.47 (s, 2H); 2.02 (s, 3H); 1.68 (s, 4H); four protons are missing because covered by the DMSO signal. HRMS (ESI) *m*/*z* calcd for $C_{26}H_{25}ClF_3N_4$ [M+H]$^+$: 485.17144; found: 485.17221. Anal. Calcd for $C_{26}H_{24}ClF_3N_4$: C, 64.39; H, 4.99; N, 11.55. Found: C, 64.51; H, 5.29; N, 10.34.

5.6.4. 7-Chloro-4-[*N*-[2-methyl-3-((diethylamino)methyl)-5-*p*-tolyl-1*H*-pyrrol-1-yl]amino]quinoline (6a)

CC (CH_2Cl_2/MeOH; 97.5:2.5); solid rinsed with a mixture of diethyl ether/petroleum ether (50:50). Yield: 40%. Mp 219–221 °C. ^{1}H NMR (DMSO-d_6): δ 10.60 (s, 1H); 8.37 (d, J = 5.23 Hz, 1H); 8.3 (d, J = 9.07, 1H); 7.87 (s, 1H); 7.59 (d, J = 8.80 Hz, 1H); 7.39 (d, J = 7.98 Hz, 2H); 7.00 (d, J = 7.98 Hz, 2H); 6.32 (s, 1H); 5.56 (d, J = 4.95 Hz, 1H); 3.29 (s, 2H); 2.14 (s, 3H); 2.02 (s, 3H); 1.01 (t, J = 6.6 Hz, 6H); four protons are missing because covered by the DMSO signal. HRMS (ESI) *m*/*z* calcd for $C_{26}H_{30}ClN_4$ [M+H]$^+$: 433.21535; found: 433.21637. Anal. Calcd for $C_{26}H_{29}ClN_4$ H_2O: C, 69.24; H, 6.93; N, 12.42. Found: C, 69.39; H,7.33; N, 12.43.

5.6.5. 7-Chloro-4-[*N*-[2-methyl-3-(pyrrolidin-1-ylmethyl)-5-*p*-tolyl-1*H*-pyrrol-1-yl]amino]quinoline (6b)

CC (CH_2Cl_2/MeOH; 96:4); solid rinsed with diethyl ether. Yield: 20%. Mp 190–191 °C. ^{1}H NMR (DMSO-d_6): δ 10.57 (s, 1H); 8.4 (d, J = 4.95 Hz, 1H); 8.34 (d, J = 9.07 Hz, 1H); 7.87 (s, 1H); 7.6 (d, J = 9.07 Hz, 1H); 7.38 (d, J = 7.98 Hz, 2H); 7.0 (d, J = 7.98 Hz, 2H); 6.31 (s, 1H); 5.57 (d, J = 4.95 Hz, 1H); 3.45 (s, 2H); 2.48 (s, 3H); 2.47 (s, 3H); 1.67 (s, 4H); four protons are missing because covered by the DMSO signal. HRMS (ESI) *m*/*z* calcd for $C_{26}H_{28}ClN_4$ [M+H]$^+$: 431.19970; found: 431.20047. Anal. Calcd for $C_{26}H_{27}ClN_4$: C, 72.46; H, 6.31; N, 13.00. Found: C, 72.40; H, 6.44; N, 12.64.

5.6.6. 7-Chloro-4-[*N*-[3-(5-(4-chlorophenyl)-3-((diethylamino)-methyl)-2-methyl-1*H*-pyrrol-1-yl)propyl]amino]quinoline (8a)

CC (CH_2Cl_2/MeOH; 93.5:6.5); solid rinsed with diethyl ether. Yield: 35%. Mp 99.3–101.2 °C. ^{1}H NMR (DMSO-d_6): δ 8.32 (d, J = 5.22 Hz, 1H); 8.08 (d, J = 9.07 Hz, 1H); 7.76 (d, J = 2.20 Hz, 1H); 7.39 (dd, J = 2.20, 9.07 Hz, 1H); 7.22 (d, J = 8.26 Hz, 2H); 7.15 (br s, 1H); 7.08 (d, J = 8.25 Hz, 2H); 6.20 (d, J = 5.50 Hz, 1H); 5.97 (s, 1H); 4.03 (t, J = 7.43 Hz, 2H); 3.30 (s, 2H); 3.10–2.95 (m, 2H); 2.39 (q, J = 6.88 Hz, 4H); 2.18 (s, 3H); 1.81 (t, J = 6.61 Hz, 2H); 0.92 (t, J = 6.88 Hz, 6H). HRMS (ESI) *m*/*z* calcd for $C_{28}H_{33}Cl_2N_4$ [M+H]$^+$: 495.20768; found: 495.20794. Anal. Calcd for $C_{28}H_{32}Cl_2N_4$: C, 67.87; H, 6.50; N, 11.31. Found: C, 67.61; H, 6.85; N, 11.26.

5.6.7. 7-Chloro-4-[*N*-[3-(5-(4-chlorophenyl)-3-(pyrrolidin-1-yl-methyl)-2-methyl-1*H*-pyrrol-1-yl)propyl]amino]quinoline (8b)

CC (CH_2Cl_2/MeOH; 95:5); solid rinsed with diethyl ether. Yield: 38%. Mp 157–158.5 °C. ^{1}H NMR (DMSO-d_6): δ 8.32 (d, J = *5.22* Hz, 1H); 8.08 (d, J = 9.07 Hz, 1H); 7.76 (s, 1H); 7.40 (d, J = 9.07 Hz, 1H); 7.22 (d, J = 8.26 Hz, 2H); 7.13 (br s, 1H); 7.08 (d, J = 8.25 Hz, 2H); 6.21 (d, J = 5.50 Hz, 1H); 5.97 (s, 1H); 4.10–3.90 (m, 2H); 3.33 (s, 2H); 3.10–2.95 (m, 2H); 2.37 (s, 4H); 2.18 (s, 3H); 1.90–1.75 (m, 2H); 1.61 (s, 4H). HRMS (ESI) *m*/*z* calcd for $C_{28}H_{31}Cl_2N_4$ [M+H]$^+$: 493.19203; found: 437.19274. Anal. Calcd for $C_{28}H_{30}Cl_2N_4$: C, 68.15; H, 6.13; N, 11.35. Found: C, 68.15; H, 6.48; N, 11.29.

5.7. 1-((7-Chloroquinolin-4-yl)amino)-2-methyl-5-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylic acid (25)

In a round bottom flask, LiOH monohydrate (1.11 g, 26.4 mmol) was dissolved in 24 ml of ethanol/water (2:1) and ester **19** (800 mg, 1.76 mmol) was added. The resulting mixture was heated at reflux for 5 h. Then the ethanol was evaporated, water was added and the resulting mixture was acidified with 1 M HCl to pH 4. The pure acid (**25**) was filtered, washed with water and dried overnight in vacuo ($CaCl_2$) and than washed with diethyl ether. Yield: 96%. Mp 205–207.5 °C (dec). ^{1}H NMR (CD_3OD): δ 8.37 (d, J = 8.8 Hz, 1H); 8.07 (d, J = 8.81 Hz, 2H); 7.93 (d, J = 6.33 Hz, 1H); 7.80 (d, J = 9.07 Hz, 2H); 7.69 (d, J = 1.65 Hz, 1H); 7.54 (dd, J = 1.92, 9.07 Hz, 1H); 7.08 (s, 1H); 5.65 (d, J = 6.6 Hz, 1H); 2.43 (s, 3H).

5.8. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-(4-nitrophenyl)-1*H*-pyrrole-3-(*N*-substituted)carboxamide (26a, 26b): general method

In a microwave vial, acid **25** (1.83 g, 4.25 mmol), HOBt (781 mg, 5.10 mmol) and appropriate amine (5.10 mmol) were dissolved in 20 ml of dry DMF at room temperature. DCC (1.05 g, 5.10 mmol) was added and the mixture was heated with a microwaves synthesizer system at 60 °C for 120 min. After cooling, the precipitate of dicyclohexylurea was filtered off and washed with DMF. The solvent was removed under vacuum and the resulting sticky solid was suspended in water, alkalinized with 2 N NaOH, filtered, washed with water and dried in a desiccator over anhydrous $CaCl_2$ (overnight under vacuum). The crude solid was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.8.1. 1-((7-Chloroquinolin-4-yl)amino)-2-methyl-5-(4-nitrophenyl)-1*H*-pyrrole-3-(*N,N*-diethyl)-carboxamide (26a)

CC (CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether. Yield: 74%. Mp 276–278.3 °C. ^{1}H NMR (DMSO-d_6): δ 11.3 (s, 1H); 8.43 (d, *J* = 7.7 Hz, 1H); 8.04 (d, *J* = 8.8 Hz, 2H); 7.89–7.86 (m, 3H); 7.86–7.41 (m, 2H); 6.79 (s, 1H); 5.35 (s, 1H); 3.44 (d, *J* = 6.88 Hz, 4H); 2.05 (s, 3H); 1.13 (t, *J* = 6.87 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{25}H_{25}ClN_5O_3$ [M+H]$^+$: 478.16404; found: 478.16410.

5.8.2. [1-((7-Chloroquinolin-4-yl)amino)-2-methyl-5-(4-nitrophenyl)-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (26b)

CC (CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether. Yield: 84%. Mp 314.5–316.5 °C. ^{1}H NMR (DMSO-d_6): δ 11.60 (s, 1H); 8.51–8.44 (m, 1H); 8.31–7.85 (m, 4H); 7.67–7.39 (m, 3H); 7.06–6.99 (m, 1H); 5.69–5.15 (m, 1H); 3.44 (br s, 4H); 2.15 (s, 3H); 1.85 (s, 4H). HRMS (ESI) *m/z* calcd for $C_{25}H_{23}ClN_5O_3$ [M+H]$^+$: 476.14839; found: 476.14847.

5.9. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-(4-aminophenyl)-1*H*-pyrrole-3-(N-substituted)carboxamide (27a, 27b): general method

Iron powder (223 mg, 4 mmol), freshly washed with 1 N HCl and distilled water, was added to a stirred solution of the corresponding nitroderivative (**26a** and **26b**; 1 mmol) in 11 ml of ethanol/water (10:1), and then 0.3 ml of glacial acetic acid was added. The suspension was heated at 80 °C with vigorous stirring under N_2 atmosphere until reaction was completed. After cooling, iron was filtered off through a pad of Celite® and washed with ethanol. After evaporation of the solvent, the residue was dissolved in a solution of CH_2Cl_2/MeOH (10:1), washed with cold 2 N NaOH and filtered off. Organic phase was dried with anhydrous Na_2SO_4, evaporated and resulting crude solid was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.9.1. 5-(4-Aminophenyl)-1-((7-chloroquinolin-4-yl)amino)-2-methyl-1*H*-pyrrole-3-(*N,N*-diethyl) carboxamide (27a)

CC (CH_2Cl_2/MeOH; 97.5:2.5); solid rinsed with diethyl ether. Yield: 71%. Mp 243.5–245.5 °C. ^{1}H NMR (DMSO-d_6): δ 10.56 (s, 1H); 8.41 (s, 1H); 8.28 (d, *J* = 7.7 Hz, 1H); 7.89 (s, 1H); 7.6 (d, *J* = 6.87 Hz, 1H); 7.15 (d, *J* = 8.26 Hz, 2H); 6.37 (d, *J* = 8.25 Hz, 2H); 6.20 (s, 1H); 5.64 (s, 1H); 5.03 (s, 2H); 3.45–3.41 (m, 4H); 3.47 (s, 3H); 1.12 (t, *J* = 6.6 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{25}H_{27}ClN_5O$ [M+H]$^+$: 448.18986; found: 448.18964.

5.9.2. [5-(4-Aminophenyl)-1-[(7-chloroquinolin-4-yl)amino]-2-methyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (27b)

CC (CH_2Cl_2/MeOH; 97:3); solid rinsed with diethyl ether. Yield: 72%. Mp 293.8–295.3 °C. ^{1}H NMR (DMSO-d_6): δ 10.56 (s, 1H); 8.40 (d, *J* = 5.22 Hz, 1H); 8.27 (d, *J* = 9.36 Hz, 1H); 7.89 (s, 1H); 7.6 (d, *J* = 8.80 Hz, 1H); 7.15 (d, *J* = 8.53 Hz, 2H); 6.42 (s, 1H); 6.36 (d, *J* = 8.25 Hz, 2H); 5.65 (d, *J* = 4.95 Hz, 1H); 5.03 (s, 2H); 3.43 (s, 2H); 3.37 (s, 2H); 2.16 (s, 3H); 1.84 (s, 4H). HRMS (ESI) *m/z* calcd for $C_{25}H_{25}ClN_5O$ [M+H]$^+$: 446.17421; found: 446.17407.

5.10. 7-Chloro-4-[*N*-[2-methyl-5-(4-aminophenyl)-3-[(N-substituted-amino)-methyl]-1*H*-pyrrol-1-yl]-amino]quinoline (7a and 7b): general method

Each amido compounds **27a** and **27b** (1 mmol) were suspended in 30 ml of anhydrous THF under N_2 and $LiAlH_4$ (296 mg, 7.8 mmol) was carefully added; the mixture was heated at reflux and stirred for 8 h and then left at 40 °C overnight. After cooling, water and 1 N NaOH was added carefully, the suspension filtrated and the residue was washed twice with THF. After evaporation of the organic solvent, the residual aqueous mixture was extracted three times with CH_2Cl_2; organic layers were dried (Na_2SO_4) and evaporated. The crude solid was purified by CC (silica gel; CH_2Cl_2 and MeOH as indicated for each single compound).

5.10.1. 7-Chloro-4-[*N*-[2-methyl-5-(4-aminophenyl)-3-((diethylamino)methyl)-1*H*-pyrrol-1-yl]-amino]quinoline (7a)

CC (CH_2Cl_2/MeOH; 98:2); solid crystalizzed and rinsed with CH_2Cl_2. Yield: 7%. Mp 250.5–254.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.49 (s, 1H); 8.00 (s, 2H); 7.81 (d, *J* = 8.25 Hz, 1H); 7.41 (d, *J* = 8.25 Hz, 1H); 7.11 (d, *J* = 7.97 Hz, 2H); 6.47 (d, *J* = 7.98 Hz, 2H); 6.25 (s, 1H); 5.99 (s, 1H); 3.58 (br s, 2H); 3.51 (s, 2H); 2.61 (d, *J* = 6.7 Hz, 4H); 1.11 (t, *J* = 6.7 Hz, 6H). HRMS (ESI) *m/z* calcd for $C_{25}H_{29}ClN_5$ [M+H]$^+$: 434.21060; found: 434.21080. Anal. Calcd for $C_{25}H_{28}ClN_5$: C, 69.19; H, 6.50; N, 16.14. Found: C, 69.26; H, 6.97; N, 16.20.

5.10.2. 7-Chloro-4-[*N*-[2-methyl-5-(4-aminophenyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-pyrrol-1-yl]-amino]quinoline (7b)

CC performed twice (CH_2Cl_2/MeOH; 96:4); solid crystalizzed and rinsed with CH_2Cl_2. Yield: 7%. Mp 216–218.5 °C (dec). ^{1}H NMR (DMSO-d_6): δ 10.46 (s, 1H); 8.38 (d, *J* = 4.95 Hz, 1H); 8.30 (d, *J* = 9.07 Hz, 1H); 7.87 (s, 1H); 7.6 (d, *J* = 8.8 Hz, 1H); 7.13 (d, *J* = 8.26 Hz, 2H); 6.35 (d, *J* = 8.28 Hz, 2H); 6.10 (s, 1H); 5.6 (s, 1H); 4.95 (s, 2H); 3.41 (s, 2H); 2.43 (s, 4H); 1.98 (s, 3H); 1.67 (s, 4H). HRMS (ESI) *m/z* calcd for $C_{25}H_{27}ClN_5$ [M+H]$^+$: 432.19495; found: 432.19501. Anal. Calcd for $C_{25}H_{26}ClN_5 \cdot 0.75H_2O$: C, 67.40; H, 6.22; N, 15.72. Found: C, 67.49; H, 6.80; N, 15.60.

5.11. Parasite cultures and drug susceptibility assay

P. falciparum cultures were carried out according to Trager and Jensen with slight modifications.[30] The CQ-sensitive, strain D10 and the CQ-resistant, strain W2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone, Celbio) medium with the addition of 1% AlbuMax (Invitrogen, Milan, Italy), 0.01% hypoxantine, 20 mM Hepes, and 2 mM glutammine. All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Compounds were dissolved in either water or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, which is non-toxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitaemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD650) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of the method of Makler in control and drug-treated cultures.[18] The antimalarial activity is expressed as 50% inhibitory concentrations (IC_{50}); each IC_{50} value is the mean and standard deviation of at least three separate experiments performed in duplicate.[31]

5.12. Cell cytotoxicity assays

The long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV 40 large T antigen[32] was maintained in MCDB 131 medium (Invitrogen, Milan, Italy) supplemented with 10% fetal calf serum (HyClone, Celbio, Milan, Italy), 10 ng/ml of epidermal growth factor (Chemicon), 1 μg/ml of hydrocortisone, 2 mM glutamine, 100 U/ml of penicillin, 100 l μg/ml of streptomycin, and 20 mM Hepes buffer (EuroClone). K562 human erythroleukemia cells and WEHI Clone 13 murine fibrosarcoma line were cultured in RPMI 1640 supplemented with 2 mM glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal calf serum. Human dermal fibroblasts (HDF) were maintained in DMEM medium (EuroClone) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin. Unless stated otherwise, all reagents were from Sigma Italia, Milan, Italy. For the cytotoxicity assays, cells were treated with serial dilutions of test compounds and cell proliferation evaluated using the MTT assay already described.[19] Plates were incubated for 72 h at 37 °C in 5% CO_2, then 20 μL of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (M-2128 Sigma) in PBS was added for an additional 3 h at 37 °C. The plates were then centrifuged, the supernatants discarded and the dark blue formazan crystals dissolved using 100 μL of lysing buffer consisting of 20% (w/v) of a solution of SDS (Sigma), 40% of *N*,*N*-dimethylformamide (Merck) in H_2O, at pH 4.7 adjusted with 80% acetic acid. The plates were then read on a microplate reader (Molecular Devices Co., Menlo Park, CA, USA) at a test wavelength of 550 nm and a reference wavelength of 650 nm. The results are expressed as IC_{50}, which is the dose of compound necessary to inhibit cell growth by 50%. All the tests were performed in triplicate at least three times.

5.13. Determination of physicochemical properties

The pK_a values of the studied compounds were determined potentiometrically at 25 °C using a PCA 101 instrument from Sirius Analytical Instruments (East Sussex, United Kingdom). Compounds were dissolved in 0.15 M KCl and the resulting solutions acidified to pH 1.8 with 0.5 M HCl. Titrations were done in triplicate with 0.5 M KOH. Partition coefficients of the compounds in *n*-octanol–0.15 M KCl were determined potentiometrically using the same instrument with three different volume ratios of organic and aqueous phases (0.2, 0.4, and 0.6). The pK_a, $\log P_{\text{Octanol}}$, and $\log D$ (distribution coefficient, defined as the ratio of the sum of concentrations for all solute species in the *n*-octanol phase to the same of the aqueous phase at selected pH) values were calculated from the titration curves using the Refinement Pro software program, v. 1.0 (Sirius Analytical Instruments). Alternatively, $\log D$ values were calculated using the following equation: $\log D = \log P - \log[1 + 10^{(pKa1-pH)} + 10^{(pKa1+pKa2-2pH)} + 10^{(pKa1+pKa2+pKa3-3pH)}]$.[33] Vacuolar accumulation ratios (VAR) and lipid accumulation ratios (LAR) were calculated as described previously.[21]

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References and notes

1. WHO. The World Health Organization—World Malaria Report 2008.
2. Guidelines for the treatment of malaria, 2nd ed. World Health Organization, Geneva, 2010.
3. Dondorp, A. M.; Yeung, S.; White, L.; Nguon, C.; Day, N. P.; Socheat, D.; von Seidlein, L. *Nat. Rev. Microbiol.* **2010**, *8*, 272.
4. Casagrande, M.; Basilico, N.; Parapini, S.; Romeo, S.; Taramelli, D.; Sparatore, A. *Bioorg. Med. Chem.* **2008**, *16*, 6813.
5. Clark, R. D.; Nelson, J. T.; Repke, D. B. *J. Heterocycl. Chem.* **1993**, *30*, 829.
6. Basha, A.; Lipton, M.; Wainrel, S. M. *Tetrahedron Lett.* **1977**, *18*, 4171.
7. Gerona-Navarro, G.; Bonacke, M. A.; Alias, M.; Perez de Vega, M. J.; Garcia-Lopez, M. T.; Lopez, P.; Cantinela, C.; Gonzales-Muniz, R. *Tetrahedron Lett.* **2004**, *45*, 2193.
8. Kuwano, R.; Takahoshi, M.; Jto, Y. *Tetrahedron Lett.* **1998**, *39*, 1017.
9. LoVerme, J.; Duranti, A.; Tontini, A.; Spadoni, G.; Mor, M.; Rivara, S.; Stella, N.; Xu, C.; Tarzia, G.; Piomelli, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 639.
10. Jung, M.; Brosch, G.; Kolle, D.; Scherf, H.; Gerhaeuser, C.; Loidl, P. *J. Med. Chem.* **1999**, *42*, 4669.
11. Gediya, L. K.; Chopra, P.; Purushottamachar, P.; Maheshwari, N.; Njar, V. C. O. *J. Med. Chem.* **2005**, *48*, 5047.
12. Glennon, R. A.; Dukat, M.; El Bermawy, M.; Law, H.; De Los Angeles, J.; Teitler, M.; King, A.; Herrich-Davis, K. *J. Med. Chem.* **1994**, *37*, 1929.
13. Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Howalter, H. D. H.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 1380.
14. Solomon, V. R.; Puri, S. K.; Srivastava, K.; Katti, S. B. *Bioorg. Med. Chem.* **2005**, *13*, 2157.
15. Scalzo, M.; Porretta, G. C.; Chimenti, F.; Casanova, M. C. *Farmaco, Ed. Sci.* **1988**, *43*, 665.
16. Bijev, A.; Yaneva, D.; Bocheva, A.; Stoev, G. *Arzneimittel Forschung* **2006**, *56*, 753.
17. Deng, S.; Wu, M.; Turtle, E. D.; Ho, W.; Arend, M. P.; Cheng, H.; Flippin, L. WO 2007115315.
18. Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739.
19. D'Alessandro, S.; Gelati, M.; Basilico, N.; Parati, E. A.; Haynes, R. K.; Taramelli, D. *Toxicology* **2007**, *241*, 66.
20. Parapini, S.; Basilico, N.; Pasini, E.; Egan, T. J.; Olliaro, P.; Taramelli, D. *Exp. Parasitol.* **2000**, *96*, 249.
21. Warhurst, D. C.; Craig, J. C.; Adagu, I. S.; Meyer, D. J.; Lee, S. Y. *Malar. J.* **2003**, *2*, 26.
22. Kuhn, Y.; Rohrbach, P.; Lanzer, M. *Cell. Microbiol.* **2007**, *9*, 1004.
23. Egan, T. J.; Chen, J. Y.; de Villiers, K. A.; Mabotha, T. E.; Naidoo, K. J.; Ncokazi, K. K.; Langford, S. J.; McNaughton, D.; Pandiancherri, S.; Wood, B. R. *FEBS Lett.* **2006**, *580*, 5105.
24. Pisciotta, J. M.; Coppens, I.; Tripathi, A. K.; Scholl, P. F.; Shuman, J.; Bajad, S.; Shulaev, V.; Sullivan, D. J., Jr. *Biochem. J.* **2007**, *402*, 197.
25. Warhurst, D. C.; Craig, J. C.; Adagu, I. S.; Guy, R. K.; Madrid, P. B.; Fivelman, Q. L. *Biochem. Pharmacol.* **2007**, *73*, 1910.
26. O'Neill, P. M.; Shone, A. E.; Stanford, D.; Nixon, G.; Asadollahy, E.; Park, B. K.; Maggs, J. L.; Roberts, P.; Stocks, P. A.; Biagini, G.; Bray, P. G.; Davies, J.; Berry, N.; Hall, C.; Rimmer, K.; Winstanley, P. A.; Hindley, S.; Bambal, R. B.; Davis, C. B.; Bates, M.; Gresham, S. L.; Brigandi, R. A.; Gomez-de-Las-Heras, F. M.; Gargallo, D. V.; Parapini, S.; Vivas, L.; Lander, H.; Taramelli, D.; Ward, S. A. *J. Med. Chem.* **2009**, *52*, 1828.
27. Kubinyi, H.. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; Wiley: New York, 1995; Vol. 1, pp 497–571.
28. Omodeo-Salè, F.; Cortelezzi, L.; Basilico, N.; Casagrande, M.; Sparatore, A.; Taramelli, D. *Antimicrob. Agents Chemother.* **2009**, *53*, 4339.
29. Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531.
30. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
31. Sparatore, A.; Basilico, N.; Parapini, S.; Romeo, S.; Novelli, F.; Sparatore, F.; Taramelli, D. *Bioorg. Med. Chem.* **2005**, *13*, 5338.
32. Ades, E. W.; Candal, F. J.; Swerlick, R. A.; George, V. G.; Summers, S.; Bosse, D. C.; Lawley, T. J. *J. Invest. Dermatol.* **1992**, *99*, 683.
33. Van de Waterbeemd, H.; Testa, B. *Adv. Drug Design* **1987**, *16*, 85.