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Novel amodiaquine congeners as potent antimalarial agents

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ABSTRACT

To develop new classes of antimalarial agents, the possibility of replacing the phenolic ring of amodiaquine, tebuquine, and isoquine with other aromatic nuclei was investigated. Within a first set of pyrrole analogues, several compounds displayed high activity against both D10 (CQ-S) and W-2 (CQ-R) strains of *Plasmodium falciparum*. The isoquine structure was also modified by replacing the diethylamino group with more metabolically stable bicyclic moieties and by replacing the aromatic hydroxyl function with a chlorine atom. Among these compounds, two quinolizidinylmethylamino derivatives (**6f** and **7f**) displayed high activity against both CQ-S and CQ-R strains.

1. Introduction

Novel, effective, safe, and inexpensive antimalarial agents are required for the management of malaria in tropical and subtropical regions, where this disease afflicts approximately 500 million people annually.[1,2]

Presently, the most promising and so far successful strategy in fighting malaria is a combination chemotherapy (ACT), in which an artemisinin derivative is used together with a conventional antimalarial drug to improve efficacy and delay onset of resistance.[1]

Despite the worldwide diffusion of resistance of *Plasmodium falciparum* to chloroquine (CQ), the 4-aminoquinoline derivatives continue to attract interest because the resistance seems to be compound specific and not related to changes in the structure of the drug target.

Indeed several CQ analogues, bearing different basic moieties, retain potent activity against CQ-resistant (CQ-R) strains of *P. falciparum.*

Also, amodiaquine (AQ) (**1**, R = H, Fig. 1) is active against many CQ-R strains of *P. falciparum*, but its clinical use has been severely restricted due to hepatotoxicity and agranulocytosis associated with long-term prophylactic use. The situation changed in the recent years with the use of AQ in association with sulfadoxine/pyrimethamine (SP) or artesunate as first line treatment for uncomplicated *P. falciparum* malaria in different African countries.[3] The efficacy, safety, and tolerability of a short-term treatment of AQ alone or AQ/SP combination in pregnant women has recently been demonstrated.[4]

From the amodiaquine scaffold, tebuquine (**2**, R = OH) was developed,[5] which resulted more active than CQ and AQ, but again chronic toxicity was seen.[6]

Since toxicity of AQ and tebuquine is related with the possibility to undergo in vivo oxidation to reactive quinoneimine derivatives, the structures of these drugs were modified in order to prevent this kind of metabolic activation. Thus, in a number of analogues the hydroxyl group was either suppressed (**2**, R = H)[7] or replaced with a fluorine atom (**1** and **2**, R = F),[7,8] or shielded with an aliphatic chain (linear or branched) in *ortho*-position (**3**)[6,9] to hinder or slow down its oxidation. Moreover in most analogues the terminal diethylamino group, that characterizes both CQ and AQ, was replaced with a cyclic basic head or with a *tert*-butylamino group in order to prevent also the side chain metabolization, a process that produces *N*-dealkyl metabolites that are less potent against CQ-R strains. All these analogues resulted more resistant to metabolic oxidation and maintained the antimalarial efficacy.

From the SAR studies, the aromatic hydroxyl function of these compounds appears to be important for increasing the antimalarial activity. Therefore, in another set of analogues, the oxidation to toxic quinoneimine metabolites was prevented by the interchange of the positions of the basic head and the hydroxyl group, leading to isoquine (**4**).[10] This compound displayed potent antimalarial activity against the CQ-S and CQ-R strains of *P. falciparum*; in particular, against the CQ-R, K-1 strain isoquine was 10 or 30 times more active than CQ, when tested as free base or diphosphate, respectively. Isoquine exhibited also excellent oral activity in mice infected with *Plasmodium yoelii* NS strain.

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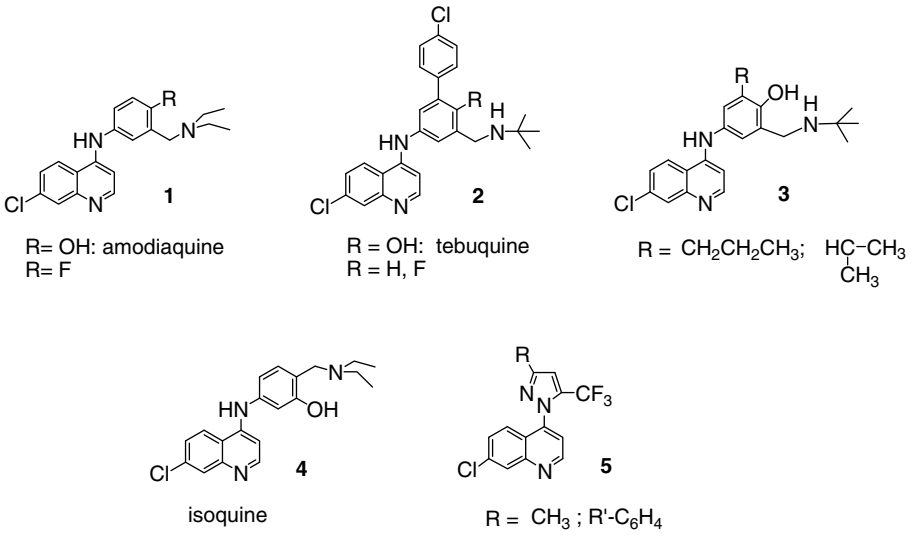


Figure 1.

Recently, we have synthesized new 4-aminoquinoline derivatives endowed with a high activity against CQ-S and CQ-R strains of *P. falciparum* and we have demonstrated that the presence of a bulky, strongly basic, and lipophilic bicyclic moiety (such as the quinolizidine or the pyrrolizidine ring), which is not supposed to be easily metabolized, appears an interesting structural feature to overcome resistance.[11,12]

Pursuing our research, we explored and here we report the effect of replacing the diethylamino group of isoquine with the bulky (pyrrolizidin-7a-yl)alkylamino and (quinolizidin-1α-yl)methylamino moieties (compounds **6d–f**, Fig. 2) that were present in our previous highly active CQ analogues. We reasoned that the high lipophilicity of these substituents could improve the cellular permeation, while the presence of an additional protonable nitrogen atom should promote the endocellular accumulation of the drug.

To further analyze the importance of the aromatic hydroxyl function, the hydroxyl group of compound **6f** was replaced by a chlorine atom (**7f**). Indeed, the 4′-dehydroxy-4′-fluoroamodiaquine,[8] while resulting more resistant to oxidation, was shown to maintain the same level of activity of AQ against CQ-S and CQ-R strains of *P. falciparum*.

We decided also to investigate the possibility to replace the benzene ring of AQ and of its analogues with other aromatic nuclei. Recently, Cunico et al.[13] described a set of 4-(pyrazol-1-yl)-7-chloroquinoline derivatives (**5**, Fig. 1), that were poorly active against the W-2 CQ-resistant strain of *P. falciparum*, with IC_{50} = 13.8 μM for the best compound (**5**, R = CH_3). In these compounds, that lack any basic head, the pyrazole ring is linked to the quinoline nucleus without the usual intercalating NH group. This supports the claim that the resonance between the nitrogen atoms of the imino group and of the quinoline is fundamental to explain the association of CQ-like drugs with ferriprotoporphyrin IX, and hence their antimalarial activity.[14]

On this ground, we managed to replace the benzene ring of AQ-like drugs with a pyrrole nucleus, still linked to the quinoline moiety through the usual NH group. The pyrrole ring retains the high reactivity of phenol, resulting well suitable for the Mannich reaction used to introduce different types of basic heads (**8**–**10**, Fig. 2).

2. Chemistry

The isoquine analogues **6d–f** (Fig. 2) were prepared in three steps as indicated in Scheme 1. The proper amine (free base or dihydrochloride) was reacted with formaldehyde and 3-acetamidophenol in absolute ethanol; the acetamido derivative was hydrolized with 20% HCl and the resulting amine was heated with 4,7-dichloroquinoline in ethanol.

Alternatively, in order to spare the costly bicyclic amines, these compounds could be prepared by reacting first 4,7-dichloroquino-

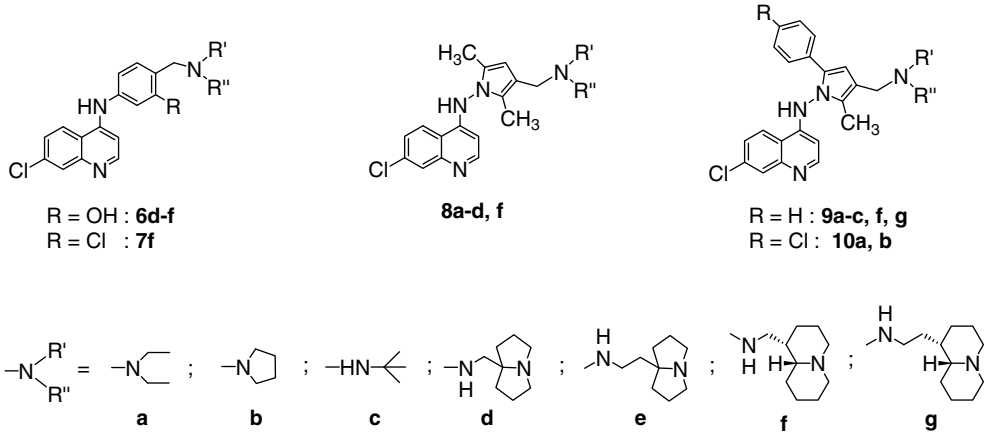
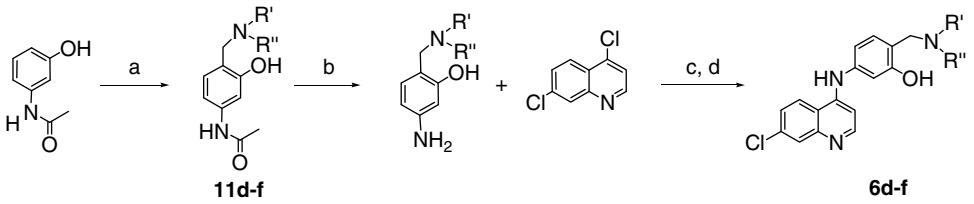


Figure 2. Structures of the investigated compounds.



Scheme 1. Reagents and conditions: (a) CH_2O, HNR′R″, EtOH, reflux, 24 h; (b) 20% HCl, reflux, 8 h; (c) EtOH, reflux, 8 h; (d) concd NH_3, pH 8.

line with 3-aminophenol, in the presence of KI,[15] and then condensing the 7-chloro-4-(3-hydroxyphenylamino)quinoline (**12**) with formaldehyde and the proper amine (Scheme 2).

Compound **7f** was obtained by coupling 2-chloro-4-nitrobenzoic acid with lupinylamine in the presence of DCC; amido and nitrogroup were reduced, respectively, with BH_3 in THF and with iron in acetic acid.[16,17] Finally, the aromatic aminocompound was reacted as usual with 4,7-dichloroquinoline (Scheme 3).

The 4-(pyrrol-1-yl)aminoquinoline derivatives **8** (Fig. 2) were obtained in two steps. First, the 7-chloro-4-hydrazinoquinoline was condensed with 2,5-hexanedione or 1-phenyl-1,4-pentandione in acetic acid, giving rise through a Paal–Knorr reaction to a pyrrole derivative, which was reacted with formaldehyde and the proper amine in acetic acid (Scheme 4) to obtain compounds **8a**–**d**, **f**.

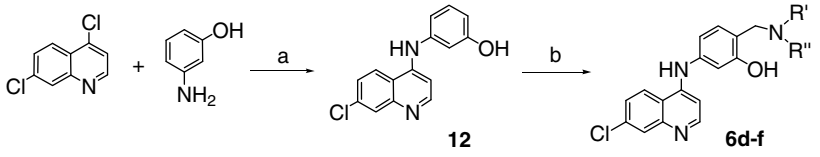
Starting from the pyrrole **17**, bearing on positions 2 and 5 different substituents, the Mannich reaction with diethylamine gave two isomeric 3- and 4-(diethylamino)methyl derivatives (as indicated by the NMR spectra) whose separation was very difficult and tedious. Thus, in this way only the 3-substituted isomer **9a** was isolated in a pure form.

Therefore, in order to obtain compounds **9** and **10**, the 7-chloro-4-hydrazinoquinoline was condensed with ethyl α-phenacyl- or α-(4-chlorophenacyl)-acetoacetate, and the obtained pyrrole derivatives (**18** and **19**) were converted to amides (**20** and **21**) by the action of the relevant amines in the presence of trimethylaluminum in toluene.[18,19] Amides were, finally, reduced to the expected aminocompounds **9a**, **b**, **f**, **g**, and **10a**, **b** by means of either lithium aluminum hydride or diphenylsilane in the presence of tris(triphenylphosphine)rhodium(I)carbonyl hydride[20,21] (Scheme 5), thus preventing the dehalogenation of the phenyl ring.

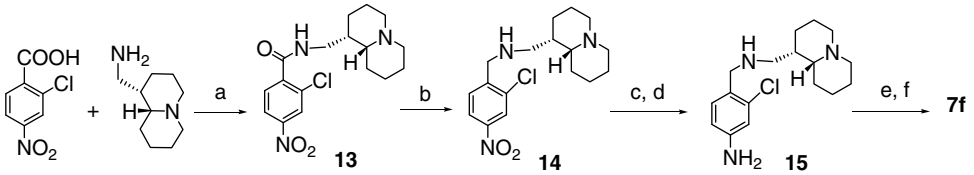
The required 1,4-diketones of Scheme 4 were commercially available, while the diketoesters of Scheme 5 were prepared according to the literature[22,23] by reacting phenacyl bromide or 4-chlorophenacyl bromide with sodium ethyl acetacetate.

The amido compound **20c**, probably due to an overwhelming steric hindrance, could not be obtained through the above described method, thus an alternative synthetic pathway was set up for compound **9c** (Scheme 6). *tert*-Butylamine was reacted with diketene to obtain N-(*tert*-butyl)acetacetamide,[24] that with phenacylbromide gave the 1,4-diketone (**22**) required to form the pyrrole derivative **20c** and then the amido compound was reduced to the amine **9c**.

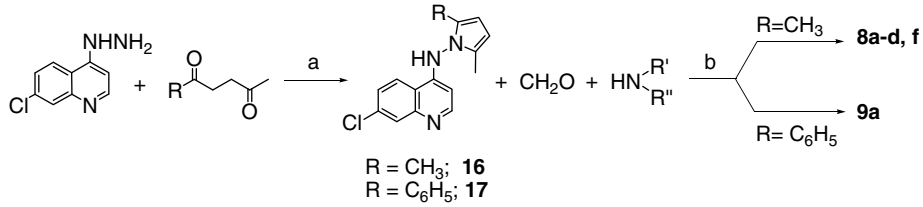
Finally, the (hexahydro-1*H*-pyrrolizin-7a-yl)methyl- and ethylamine and the (1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl- and ethylamine (lupinylamine and *homo*-lupinylamine), required to obtain compounds of Figure 2, were prepared, respectively, as previous described by Oka et al.,[25–27] Sparatore et al.,[28] and Boido et al.[29]



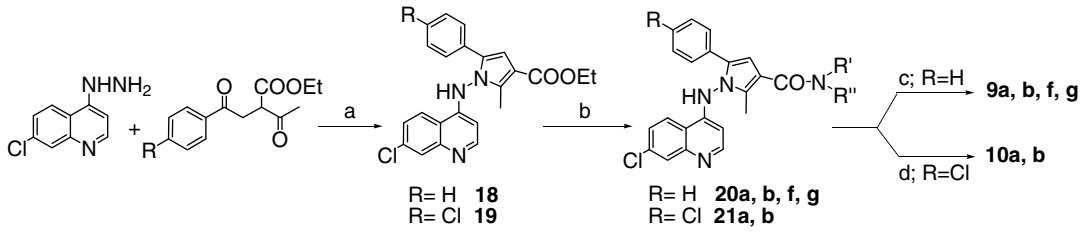
Scheme 2. Reagents and conditions: (a) 2 N HCl, KI, EtOH, reflux; (b) CH_2O, HNR′R″, EtOH, reflux.



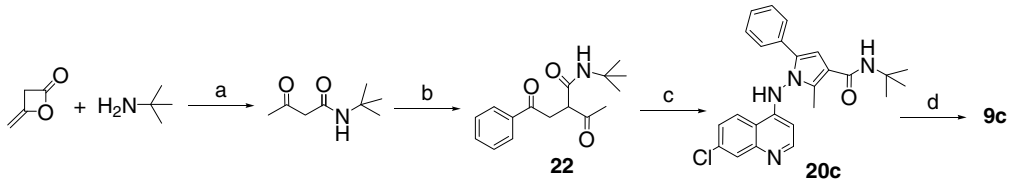
Scheme 3. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, rt; (b) BH_3–THF, THF, 0 °C to reflux; (c) Fe, AcOH, EtOH/H_2O, reflux; (d) concd NH_3; (e) 4,7-dichloroquinoline, 6 N HCl, KI, EtOH, reflux; (f) aq NaOH.



Scheme 4. Reagents and conditions: (a) AcOH, 65 °C, 3 h; (b) AcOH, rt, 2–3 h.



Scheme 5. Reagents and conditions: (a) AcOH, reflux; (b) $AlMe_3$, HNR′R″, PhMe, reflux; (c) $LiAlH_4$, Et_2O, reflux; (d) Ph_2SiH_2, $(PPh_3)_3(CO)HRh$, THF.



Scheme 6. Reagents and conditions: (a) Et_3N, CH_2Cl_2, 0° to rt; (b) phenacyl bromide, EtONa, Et_2O, reflux; (c) 7-chloro-4-hydrazinoquinoline, AcOH, reflux; (d) $LiAlH_4$, Et_2O, reflux.

Structures of most final compounds and intermediates were supported by ^1H NMR and high-resolution mass spectra; final compounds **8a** and **8b** were characterized by ^1H NMR and elemental analyses, while the intermediate **11e**, which was used without a complete purification, was characterized by ^1H NMR and low resolution mass spectrum.

3. Results and discussion

The 16 compounds of Figure 2 were tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. The antimalarial activity was quantified as inhibition of parasite growth, measured with the production of parasite lactate dehydrogenase (pLDH).[30] Cytotoxicity on mammalian cell lines was assayed using the MTT test.[31]

Table 1 shows the IC_{50} (nM) and the means of the ratios between the IC_{50} of CQ and that of each compound against D-10 or W-2 strains, calculated for each single experiment (since all the compounds were not tested simultaneously). The ratios between the IC_{50} of each compound against the two strains of *P. falciparum* are also indicated. The last value is suggestive of the susceptibility of the drug to the resistance mechanisms (resistance factor).

All tested compounds exhibited from moderate to high activity on the CQ-S (D-10) strain, with IC_{50} ranging from 8.5 to 222.2 nM. CQ IC_{50} was 27.2 nM (range 15–39 nM), thus the tested compounds were from 0.1- to 2.8-fold as active as CQ.

Five of the new compounds also exhibited a strong activity against the CQ-R (W-2) strain, resulting from 10 to 28 times more active than CQ, with IC_{50} values as low as 14–59 nM (**9a**, **10b**, **10a**, **6f**, and **7f**) compared to 481.2 nM of CQ (range 165–805 nM). Four additional compounds (**6e**, **6d**, **8f**, and **9b**) were in the order from 2- to 4.2-fold more active than the reference drug, while the remaining seven compounds were from 0.5 to 1.2 times as active as CQ on the W-2 strain.

Moreover, for the five most potent compounds the resistance factor ranged from 1.2 to 1.7, resulting about ten times lower than

Table 1
In vitro antimalarial activity against D-10 and W-2 strains of *P. falciparum* and cytotoxicity against human (HMEC-1 and K562) and murine (WEHI) cell lines of compounds **6–10**

Compound	D-10 (CQ-S) IC_{50}[a] (nM)	Ratio IC_{50} CQ/Comp.[b]	W-2 (CQ-R) IC_{50}[a] (nM)	Ratio IC_{50} CQ/Comp.[b]	Ratio IC_{50} CQ-R/CQ-S[c]	HMEC-1 IC_{50}[d] (nM)	K562 IC_{50}[d] (nM)	WEHI 13 IC_{50}[d] (nM)
6d	81.9 ± 3.4	0.4	76.96 ± 14.6	2.2	0.9	n.t.	n.t.	n.t.
6e	90.3 ± 48.5	0.3	153.1 ± 24.7	2.1	1.7	n.t.	n.t.	12,211 ± 986
6f	8.5 ± 1.9	2.8	14.3 ± 7.8	22.0	1.7	7938 ± 1242	n.t.	4427 ± 655
7f	23.6 ± 5.1	1.8	28.8 ± 4.5	28.0	1.2	5517 ± 1193	n.t.	2769 ± 1278
8a	69.0 ± 25.3	0.2	192.9 ± 4.0	1.2	2.8	n.t.	n.t.	n.t.
8b	80.7 ± 27.1	0.2	336.3 ± 64.3	0.7	4.2	n.t.	n.t.	n.t.
8c	48.6 ± 10.5	0.3	255.2 ± 53.7	0.9	5.3	n.t.	n.t.	n.t.
8d	222.2 ± 15.6	0.1	358.3 ± 20.6	0.5	1.6	n.t.	n.t.	n.t.
8f	70.7 ± 5.3	0.4	53.4 ± 16.4	3.1	0.8	5398 ± 841	n.t.	5508 ± 3827
9a	42.7 ± 8.9	0.7	59.2 ± 27.4	9.9	1.4	16,694 ± 5919	3616 ± 1268	4380 ± 2105
9b	42.9 ± 13.0	0.7	139.5 ± 56.0	4.2	3.3	12,874 ± 2824	2920 ± 866	3878 ± 638
9c	63.5 ± 18.2	0.4	1176.1 ± 538.9	0.7	18.5	n.t.	n.t.	n.t.
9f	36.5 ± 12.6	0.8	515.0 ± 252.8	1.1	14.1	13,451 ± 7111	1683 ± 394	7441 ± 1254
9g	154.1 ± 37.5	0.2	776.2 ± 386.2	0.8	5.0	14,599 ± 5031	2614 ± 629	7219 ± 1325
10a	26.1 ± 8.3	0.9	42.5 ± 19.1	18.5	1.6	5812 ± 1511	n.t.	1446 ± 670
10b	30.7 ± 4.3	0.8	49.6 ± 5.2	15.8	1.6	7176 ± 1383	n.t.	2060 ± 125
Isoquine	23.7 ± 3.0	1.3	30.2[e]	17.6	1.3	n.t.	n.t.	n.t.
CQ	27.2 ± 3.5	—	481.2 ± 183.9	—	17.7	>32,000	29,030 ± 5701	>38,000

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.

that of CQ (17.7), suggesting that they were not (or only poorly) affected by the resistance mechanisms.

With regards to the structure–activity relationships among the isoquine analogues, it has been observed that the quinolizidinylmethylamino derivative (**6f**) exhibited a 10-fold higher activity than the pyrrolizidinylalkylamino analogues **6d** and **6e**. Such a difference was unexpected, since in our previously described CQ analogues,[11,12] characterized also by the presence of these basic moieties, the pyrrolizidinylalkylamino derivatives were somewhat more active than the quinolizidinylalkylamino compounds.

Within the present series of compounds, the unsuitability of the pyrrolizidinyl moiety was further observed in compound **8d**, that was the least active among all.

Interestingly, the replacement of the hydroxyl group of **6f** with a chlorine atom (**7f**) enhanced the activity against the CQ-R strain of *P. falciparum* (ratio IC_{50}CQ/compound), further supporting the observation of O'Neill et al.[8] that the hydroxyl is useful but not absolutely necessary for activity.

O'Neill et al.[10] tested isoquine against a CQ-R strain of *P. falciparum* (K1) different from that used by us, thus not allowing a direct comparison with our compounds. Such a comparison appears even more difficult, since isoquine exhibited different degrees of activity when assayed as free base or as diphosphate salt.

Therefore, we prepared isoquine according to O'Neill et al. procedure[10] and tested it as free base against the same CQ-S and CQ-R strains used for our compounds. Thus, we observed that isoquine was 17.6 times more active than CQ on W-2 strain of *P.falciparum*, while **6f** and **7f** were, respectively, 22 and 28 times more active.

The replacement of the phenolic ring, typical of AQ, tebuquine, and isoquine, with a pyrrolic ring is associated with a good antimalarial activity. Therefore, these compounds represent a new class of antimalarial agents worthy of further investigation. Within this class of compounds the activity increased with the increasing lipophilicity; thus 2-methyl-5-(4-chlorophenyl)- and 2-methyl-5-phenyl-derivatives (**10a**, **10b**, and **9a**–**9f**, respectively), more resembling tebuquine, were more active than the corresponding 2,5-dimethyl derivatives (**8a**–**8f**). With regards to the effects of the basic heads on the activity, it is observed that the smaller diethylamino and pyrrolidino moieties are more profitable when a phenyl substituent is present on the pyrrole ring, while the bulkier quinolizidinylmethylamino group (**8f**) is advantageous within the 2,5-dimethylpyrrole subset of compounds.

The most active compounds (**6f**, **7f**, **8f**, **9a**, **10a**, and **10b**) exhibit low toxicity against three different human or mouse cell lines with a selectivity index ranging between 555 and 34.

4. Conclusions

In order to develop new classes of antimalarial agents, the possibility of replacing the phenolic ring of AQ, tebuquine, and isoquine with other kinds of aromatic nuclei was investigated.

Within a first set of pyrrole analogues, several compounds (**8f**, **9a**, **10a**, and **10b**) displayed high activity against W-2 CQ-R strain of *P. falciparum* with good selectivity indexes.

The isoquine structure was also modified by replacing the diethylamino group with some more metabolically stable bicyclic moieties. Compound (**6f**), bearing a quinolizidinylmethylamino residue, displayed high activity against both CQ-S and CQ-R strains. By replacing the aromatic hydroxyl function with a chlorine atom (**7f**), the activity ratio with CQ was further increased. On the basis of these results, the exchange of phenol ring of amodiaquine-like drugs with other aromatic moieties, as well as the replacement of the usual amino groups with pyrrolizidine and quinolizidine-derived basic heads will be further investigated.

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 with Me_4Si as internal standard; δ in parts per million, J in Hertz. Elemental analyses (of compounds **8a** and **8b**) were performed on a Carlo Erba-EA-1110 CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmaceutical Sciences of Genoa University. Mass spectra were recorded on a LCQ Advantage (Thermofinnigan) mass spectrometer and high-resolution mass spectra (HRMS) on a APEX II ICR-FTMS Bruker Daltonics mass spectrometer in positive or negative electro spray ionization (ESI).

5.2. *N*-[3-Hydroxy-4-[(*N*′-substituted-amino)methyl] phenyl]acetamide (11d–f): general method

3-Acetamidophenol (756 mg, 5 mmol) was added to a stirred solution of amine (free base, 5 mmol) and 37% aqueous formaldehyde (5 mmol, 0.37 ml) in 4 ml of absolute EtOH. The resulted solution was heated at reflux for 24 h under an atmosphere of N_2. After cooling, organic layer was evaporated and the obtained solid was purified on silica gel column (CC) using CH_2Cl_2–MeOH (with or without concd NH_3) as eluent to afford the title compounds.

5.2.1. *N*-[4-[[(Hexahydro-1*H*-pyrrolizin-7a-yl)methyl amino]methyl]-3-hydroxyphenyl]acetamide (11d)

CC (CH_2Cl_2/MeOH/concd NH_3; from 93:6.3:0.7 to 90:9:1). Amorphous solid rinsed with ethyl ether. Yield: 18%. ^{1}H NMR (DMSO-d_6): δ 9.72 (s, 1H); 7.02 (d, J = 1.92 Hz, 1H); 6.95 (d, J = 8.20 Hz, 1H); 6.85 (dd, J = 8.20, 1.92 Hz, 1H); 3.72 (s, 2H); 2.90–2.75 (m, 2H); 2.33 (s, 2H); 1.98 (s, 3H); 1.80–1.35 (m, 10H). HRMS (ESI) m/z calcd for $C_{17}H_{26}N_3O_2$ $[M+H]^+$: 304.20195; found: 304.20222.

5.2.2. *N*-[4-[[2-(Hexahydro-1*H*-pyrrolizin-7a-yl) ethylamino]methyl]-3-hydroxyphenyl]acetamide (11e)

CC (CH_2Cl_2/MeOH/concd NH_3; up to 77:22:1). Amorphous solid rinsed with a mixture of ethyl ether/CH_2Cl_2 (1:1). Yield: 14%. ^{1}H NMR ($CDCl_3$): δ 6.87 (dd, J = 8.20, 1.92 Hz, 1H); 6.62 (d, J = 8.20 Hz, 1H); 6.50 (d, J = 1.92 Hz, 1H); 3.90 (s, 2H); 3.00–2.90 (m, 2H); 2.65–2.50 (m, 2H); 2.15 (s, 3H); 1.90–1.35 (m, 12H). MS (ESI): m/z 318 $[M+H]^+$.

5.2.3. *N*-[3-Hydroxy-4-[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methylamino]methyl]phenyl]acetamide (11f)

CC (CH_2Cl_2/MeOH; 92:8). Solid rinsed with ethyl ether. Yield: 21%. Mp 117.8–122 °C. ^{1}H NMR (DMSO-d_6): δ 7.20 (s, 1H); 7.05 (dd, J = 8.20, 1.92 Hz, 1H); 6.88 (d, J = 8.20 Hz, 1H); 6.80 (d, J = 1.92 Hz, 1H); 3.90 (s, 2H); 2.90–2.70 (m, 4H); 2.10 (s, 3H); 2.10–1.40 (m, 14H). HRMS (ESI) m/z calcd for $C_{19}H_{30}N_3O_2$ $[M+H]^+$: 332.23325; found: 332.23328.

5.3. 5-[(7-Chloroquinolin-4-yl)amino]-2-[(substituted-amino) methyl]phenol (6d–f): general method

A stirred solution of the above acetamide (1 mmol) in 3 ml of 20% HCl was refluxed for 8 h under atmosphere of N_2. The solution was evaporated to dryness under vacuum, the residue was dissolved in 3 ml of absolute EtOH and 4,7-dichloroquinoline (198 mg, 1 mmol) was added. The stirred mixture was heated for 8 h under N_2. After cooling, the organic layer was evaporated, the

residue was dissolved in water, alkalized with concd NH_3 and extracted with CH_2Cl_2. The organic layer was dried on anhydrous Na_2SO_4 and evaporated; the obtained solid was purified on silica gel column (CC) using CH_2Cl_2–MeOH (with or without concd NH_3) as eluent to afford the title compounds.

5.3.1. 5-[(7-Chloroquinolin-4-yl)amino]-2-[[(hexahydro-1*H*-pyrrolizin-7a-yl)methylamino]methyl]phenol (6d)

CC (CH_2Cl_2/MeOH; up to 83:17); followed by a preparative TLC on silica gel (Kieselgel 60 F_{254}, 1 mm, CH_2Cl_2/MeOH/concd NH_3; 10:2:0.2). Solid washed with ethyl ether. Yield: 6%. Mp 109–120.5 °C. ^{1}H NMR (DMSO-d_6): δ 8.90 (s, 1H); 8.45–8.25 (m, 2H); 7.85 (s, 1H); 7.53 (d, J = 9.07 Hz, 1H); 7.07 (d, J = 9.07 Hz, 1H); 6.90 (s, 1H); 6.60–6.75 (m, 2H); 3.80 (s, 2H); 2.90–2.80 (m, 2H); 2.36 (s, 2H); 1.80–1.00 (m, 10H). HRMS (ESI) m/z calcd for $C_{24}H_{28}ClN_4O$ $[M+H]^+$: 423.19462; found: 423.19524.

5.3.2. 5-[(7-Chloroquinolin-4-yl)amino]-2-[[2-(hexahydro-1*H*-pyrrolizin-7a-yl)ethylamino]methyl]phenol (6e)

CC (CH_2Cl_2/MeOH; from 95:5 to 88:12). Solid rinsed with a mixture of ethyl ether–CH_2Cl_2. Yield: 38%. Mp 204–206.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.50 (d, J = 5.50 Hz, 1H); 8.00 (d, J = 1.93 Hz, 1H); 7.80 (d, J = 9.07 Hz, 1H); 7.40 (dd, J = 1.93, 9.08 Hz, 1H); 7.10–6.80 (m, 3H); 6.70 (dd, J = 1.92, 7.70 Hz, 1H); 4.00 (s, 2H); 3.00–2.80 (m, 2H); 2.80–2.40 (m, 4H); 1.70–1.40 (m,10H). HRMS (ESI) calcd for $C_{25}H_{30}ClN_4O$ $[M+H]^+$: 437.21027; found: 437.21110. The free base was converted to tri-hydrochloride (mp 247.2–256.7 °C) for biological tests.

5.3.3. 5-[(7-Chloroquinolin-4-yl)amino]-2-[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methylamino]methyl]phenol (6f)

CC (CH_2Cl_2/MeOH/concd NH_3; from 96.5:3.2:0.3 to 96:3.6:0.4). Solid rinsed with ethyl ether. Yield: 34%. Mp 136–137.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.53 (d, J = 5.50 Hz, 1H); 8.00 (d, J = 1.93 Hz, 1H); 7.88 (d, J = 9.07 Hz, 1H); 7.45 (dd, J = 1.93, 9.08 Hz, 1H); 7.10–6.90 (m, 2H); 6.75 (s, 1H); 6.70 (dd, J = 1.92, 7.70 Hz, 1H); 4.00 (s, 2H); 3.00–2.80 (m, 4H); 2.40–1.20 (m, 14H). HRMS (ESI) m/z calcd for $C_{26}H_{32}ClN_4O$ $[M+H]^+$: 451.22592; found: 451.22538.

5.4. 5-[(7-Chloroquinolin-4-yl)amino]-2-[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methylamino]methyl]phenol (6f): alternative method

Aq. formaldehyde (37%, 0.14 ml, 1.5 mmol) was added to a stirred solution of aminolupinane (252 mg, 1.5 mmol) in 10 ml of absolute ethanol; after a few minutes compound **12**[15] (406 mg, 1.5 mmol) and other 5 ml of ethanol were added and the mixture was heated for 6 h under N_2 atmosphere. After cooling, ethanol was evaporated and the residue was chromatographed on a column of silica gel using CH_2Cl_2/MeOH/concd NH_3 (96:3.6:0.4) as eluent. Solid rinsed with a mixture of petroleum ether/ethyl ether (90:10). Yield: 40%. Mp 139–142 °C.

5.5. 2-Chloro-4-nitro-*N*-[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methyl]benzamide (13)

To a solution of 2-chloro-4-nitrobenzoic acid (900 mg, 4.46 mmol) in 12 ml of anhydrous dichloromethane DCC (1.06 g, 5.13 mmol) and DMAP (16.5 mg, 0.134 mmol) were added. The solution was stirred at rt for 15 min, a solution of aminolupinane[28] (750 mg, 4.46 mmol) in 4 ml of anhydrous dichloromethane was slowly added dropwise and the mixture was further stirred for 4 h at rt. The precipitate was filtered off and the organic layer was washed with 2 N NaOH, then with brine, dried, and finally evaporated in vacuo.

The crude product was chromatographed on a column of silica gel (CH_2Cl_2/MeOH, from 97:3 to 92:8). The resulted solid was washed with ethyl ether. Yield: 52%. Mp 126.1–128.3. ^{1}H NMR ($CDCl_3$): δ 8.78 (s, 1H); 8.30 (s, 1H); 8.15 (d, J = 9.08 Hz, 1H); 7.75 (d, J = 9.08 Hz, 1H); 3.75–3.55 (m, 2H); 2.90–2.70 (m, 2H); 2.40–1.15 (m, 14H). HRMS (ESI) m/z calcd for $C_{17}H_{23}ClN_3O_3$ $[M+H]^+$: 352.14225; found: 352.14330.

5.6. *N*-(2-Chloro-4-nitrobenzyl)((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methanamine (14)

A solution of **13** (380 mg, 1.08 mmol) in 3 ml of anhydrous THF was added dropwise to a stirred, ice-cold solution of BH_3 in THF (1 N, 17 ml, 17 mmol). When addition was completed, the mixture was heated at reflux for 6 h under N_2. After, to the ice-cooled solution, 7 ml of 10 N HCl were carefully added and the solution was heated at reflux for 10 min. The THF was removed under vacuum; the resulting acid solution was saturated with NaOH pellets and extracted three times with CH_2Cl_2. The solvent was removed and the crude oil was purified by CC (silical gel, CH_2Cl_2/MeOH; from 98:2 to 94:6). The obtained oil was taken up in petroleum ether and, after filtration, the solvent was removed to leave a sticky oil. Yield: 87%. ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H); 8.10 (d, J = 9.08 Hz, 1H); 7.70 (d, J = 9.08 Hz, 1H); 3.95 (s, 2H); 2.95–2.65 (m, 4H); 2.25–1.00 (m, 14H). HRMS (ESI) m/z calcd for $C_{17}H_{25}ClN_3O_2$ $[M+H]^+$: 338.16298; found: 338.16309.

5.7. 3-Chloro-4-[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methylamino]methyl]benzenamine (15)

Iron powder (270 mg, 4.8 mmol), freshly washed with 1 N HCl and distilled water, was added to a stirred solution of **14** (400 mg, 1.18 mmol) in 8.7 ml of ethanol/water (2:1), to which 0.3 ml of glacial acetic acid was then added. The suspension was heated at reflux with vigorous stirring for 20 min under N_2 atmosphere. After cooling, the suspension was alkalized with concd NH_3 and filtered through a pad of Celite®. The filtrate was concentrated under vacuum, diluted with water, and extracted with ethyl acetate. The organic layer was dried and evaporated, and resulting crude oil was purified by CC (Grade III alumina; cyclohexane/ethyl acetate, 70:30) to afford compound **15** as an oil. Yield: 75%. ^{1}H NMR ($CDCl_3$): δ 7.10 (d, J = 9.05 Hz, 1H); 6.65 (s, 1H). 6.50 (d, J = 9.05 Hz, 1H); 3.78–3.50 (m, 4H); 2.90–2.55 (m, 4H); 2.18–1.00 (m, 14H). HRMS (ESI) m/z calcd for $C_{17}H_{27}ClN_3$ $[M+H]^+$: 308.18880; found: 308.18917.

5.8. 7-Chloro-4-[*N*-[3-chloro-4-[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methylamino]methyl]phenyl]amino]quinoline (7f)

To a stirred suspension of 4,7-dichloroquinoline (181 mg, 0.92 mmol) in 4 ml of EtOH 0.33 ml of 6 N HCl was added. When the 4,7-dichloroquinoline was dissolved, compound **15** (270 mg, 0.88 mmol) and KI (2 mg) were added and the mixture was stirred and heated at reflux for a night under N_2 atmosphere.

After cooling, the solvent was removed, the residue was taken up in 2 N NaOH and the mixture was extracted with ethyl acetate. The crude product was purified by CC (silica gel; CH_2Cl_2/MeOH/concd NH_3; from 92:8:0 to 90:9.5:0.5). Solid rinsed with ethyl ether. Yield: 79%. Mp 140.3–142.4 °C. ^{1}H NMR ($CDCl_3$): δ 8.60 (d, J = 5.23 Hz, 1H); 8.04 (d, J = 1.93 Hz, 1H); 7.85 (d, J = 9.08 Hz, 1H); 7.50–7.40 (m, 2H); 7.30 (d, J = 2.20 Hz, 1H); 7.15 (dd, J = 8.25, 2.20 Hz, 1H); 6.97 (d, J = 5.22 Hz, 1H); 6.65 (s, 1H); 3.85 (s, 2H); 2.90–2.75 (m, 4H); 2.15–1.35 (m, 14H). HRMS (ESI) m/z calcd for $C_{26}H_{31}Cl_2N_4$ $[M+H]^+$: 469.19203; found: 469.19200.

5.9. 7-Chloro-4-[*N*-(2,5-dimethyl-1H-pyrrol-1-yl)amino]quinoline (16)

2,5-Hexandione (767 mg, 6.72 mmol) was added to a stirred solution of 7-chloro-4-hydrazinoquinoline (1.084 g, 5.6 mmol) in glacial acetic acid (5.6 ml), and the mixture was heated at 65 °C under N_2 for 3 h. After cooling, the solution was concentrated under vacuum, the residue dissolved in 50 ml of water and alkalized with concd NH_3; the precipitate was filtrated, washed with cool water, and purified by CC (silica gel; CH_2Cl_2 containing 0.5–1% of MeOH). Then the resulting solid was rinsed with ethyl ether. Yield: 90%. Mp 226–227.5 °C. ^{1}H NMR (DMSO-d_6): δ 10.33 (s, 1H); 8.44 (d, J = 4.95 Hz, 1H); 8.31 (d, J = 9.07 Hz, 1H); 7.90 (d, J = 1.92 Hz, 1H); 7.57 (dd, J = 9.08, 1.93 Hz, 1H); 5.83 (s, 2H); 5.61 (d, J = 5.03 Hz, 1H); 1.95 (s, 6H). HRMS (ESI) m/z calcd for $C_{15}H_{15}ClN_3$ $[M+H]^+$: 272.09490; found: 272.09469.

5.10. 7-Chloro-4-[*N*-(2-methyl-5-phenyl-1*H*-pyrrol-1-yl)amino]-quinoline (17)

1-Phenyl-1,4-pentandione (846 mg, 4.8 mmol) was added to a stirred solution of 7-chloro-4-hydrazinoquinoline (774 mg, 4 mmol) in glacial acetic acid (4 ml) and the mixture was refluxed under N_2 for 2 h. After cooling, the solution was concentrated under vacuum and the residue dissolved in CH_2Cl_2; the organic phase was washed twice with 1 N NaOH, dried (Na_2SO_4), the solvent removed and the crude solid was purified by CC (silica gel; CH_2Cl_2 up to 4% of MeOH). The violet resulting solid was further purified by chromatography on grade I alumina (CH_2Cl_2/cyclohexane from 90:10 to 95:5) to give a white solid. Yield: 44%. Mp 195.5–196.2 °C. ^{1}H NMR (DMSO-d_6): δ 10.60 (s, 1H); 8.39 (d, J = 4.70 Hz, 1H); 8.30 (d, J = 9.08 Hz, 1H); 7.85 (s, 1H); 7.61 (d, J = 8.53 Hz, 1H); 7.50 (d, J = 7.70 Hz, 2H); 7.18 (t, J = 7.43 Hz, 2H); 7.07 (d, J = 7.15 Hz, 1H); 6.40 (d, J = 3.3 Hz, 1H); 6.10 (s, 1H); 5.60 (d, J = 4.40 Hz, 1H); 2.02 (s, 3H). HRMS (ESI) m/z calcd for $C_{20}H_{17}ClN_3$ $[M+H]^+$: 334.11055; found: 334.11143.

5.11. 7-Chloro-4-[*N*-[2,5-dimethyl-3-[(N-substituted)amino-methyl]-1*H*-pyrrol-1-yl]amino]quinoline (8a–d and f): general method

The proper amine (as free base or dihydrochloride; 1.5 mmol, for secondary amines; 1.65 mmol, for primary amines), aq formaldehyde (37%, 1.5 mmol, for secondary amines; 1.65 mmol, for primary amines) were added to 0.5 ml of glacial acetic acid in an ice-cooled flask. The solution was stirred for 2–3 min, and then poured into a flask containing **16** (407.6 mg, 1.5 mmol). The mixture was stirred at rt under N_2 for 2 h, water (15 ml) was added and the solution alkalized with 2 N NaOH. The resulting suspension was extracted three times with CH_2Cl_2 and the collected organic phases were dried (Na_2SO_4) and evaporated to give a crude derivative, which was purified by CC (silica gel; CH_2Cl_2–MeOH, with or without concd NH_3).

5.11.1. 7-Chloro-4-[*N*-[3-((diethylamino)methyl)-2,5-dimethyl-1*H*-pyrrol-1-yl]amino]quinoline (8a)

CC (CH_2Cl_2/MeOH/concd NH_3; from 97:2.7:0.3 to 95:4.5:0.5); solid rinsed with petroleum ether/ethyl ether (30:70). Yield: 65%. Mp 248–251 °C (dec.). ^{1}H NMR ($CDCl_3$): δ 8.51 (d, J = 4.67 Hz, 1H); 8.04 (s, 1H); 7.92 (d, J = 8.25 Hz, 1H); 7.80 (s, 1H); 7.48 (dd, J = 9.07, 1.92 Hz, 1H); 5.94 (s, 1H); 5.81 (d, J = 4.95 Hz, 1H); 3.45 (s, 2H); 2.55 (q, J = 6.88 Hz, 4H); 2.15–1.95 (m, 6H); 1.10 (t, J = 6.82 Hz, 6H). Anal. Calcd for $C_{20}H_{25}ClN_4 + H_2O$: C, 64.07; H, 7.25; N, 14.94. Found: C, 63.68; H, 7.52; N, 14.71.

5.11.2. 7-Chloro-4-[*N*-[2,5-dimethyl-3-((pyrrolidin-1-yl)methyl)-1*H*-pyrrol-1-yl]amino]quinoline (8b)

CC (CH_2Cl_2/MeOH; from 95:5 to 88:12); solid rinsed with a mixture of EtOH–ethyl ether. Yield: 64%. Mp 160.5–162.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.50 (d, J = 4.67 Hz, 1H); 8.03 (s, 1H); 7.90 (d, J = 8.25 Hz, 1H); 7.47 (dd, J = 9.08, 1.92 Hz, 1H); 5.95 (s, 1 H); 5.80 (d, J = 4.95 Hz, 1H); 3.45 (s, 2H); 2.55 (s, 4H); 2.15–1.95 (m, 6H); 1.80 (s, 4H). Anal. Calcd for $C_{20}H_{23}Cl_1N_4 + 1/2H_2O$: C, 66.01; H, 6.65; N, 15.40. Found: C, 65.84; H, 6.43; N, 15.42.

5.11.3. 7-Chloro-4-[*N*-[3-((*tert*-butylamino)methyl)-2,5-dimethyl-1*H*-pyrrol-1-yl]amino]quinoline (8c)

CC (CH_2Cl_2/MeOH/concd NH_3; from 96:3.6:0.4 to 94:5.4:0.6); the resulting solid was dissolved into a mixture of cyclohexane/dry ethyl ether (50:50), the solution was filtered and the solvent was evaporated to leave pure compound. Yield: 27%. Mp 103.5–107.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.50 (d, J = 4.67 Hz, 1H); 8.03 (s, 1H); 7.90 (d, J = 8.25 Hz, 1H); 7.70 (br s, 1H); 7.47 (dd, J = 9.08, 1.92 Hz, 1H); 5.97 (s, 1H); 5.86 (d, J = 4.95 Hz, 1H); 3.60 (s, 2H); 2.10–1.90 (m, 6H); 1.22 (s, 9H). HRMS (ESI) m/z calcd for $C_{20}H_{26}ClN_4$ $[M+H]^+$: 357.18405; found: 357.18396.

5.11.4. 7-Chloro-4-[*N*-[2,5-dimethyl-3-[[(hexahydro-1*H*-pyrrolizin-7a-yl)methylamino]methyl]-1*H*-pyrrol-1-yl]amino]quinoline (8d)

CC (CH_2Cl_2/MeOH/concd NH_3; from 96:3.6:0.4 to 94:5.4:0.6); solid rinsed with dry ethyl ether. Yield: 23%. Mp 92–97 °C (dec). ^{1}H NMR ($CDCl_3$): δ 8.56 (s, 1H); 8.45 (d, J = 4.67 Hz, 1H); 8.00 (s, 1H); 7.90 (d, J = 8.25 Hz, 1H); 7.47 (dd, J = 9.08, 1.92 Hz, 1H); 5.90 (s, 1H); 5.86 (d, J = 4.95 Hz, 1H); 3.62 (s, 2H); 3.35–3.15 (m, 2H); 3.10 (br s, 1H); 2.75–2.60 (m, 2H); 2.20–1.50 (m, 16H). HRMS (ESI) m/z calcd for $C_{24}H_{31}Cl_1N_5$ $[M+H]^+$: 424.22625; found: 424.22695.

5.11.5. 7-Chloro-4-[*N*-[2,5-dimethyl-3-[[[((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)methyl]amino]methyl]-1*H*-pyrrol-1-yl]amino]-quinoline (8f)

CC (CH_2Cl_2/MeOH/concd NH_3; from 96:3.6:0.4 to 95:4.5:0.5); solid rinsed with dry ethyl ether. Yield: 35%. Mp 188–191 °C. ^{1}H NMR ($CDCl_3$): δ 8.40 (d, J = 4.59 Hz, 1H); 8.10–7.80 (m, 2H); 7.40 (d, J = 9.08, 1H); 5.97–5.65 (m, 2H); 3.65–3.30 (m, 2H); 3.30–2.95 (m, 2H); 2.95–2.65 (m, 2H); 2.65–0.60 (m, 20H). Anal. Calcd for $C_{26}H_{34}Cl_1N_5$: C, 69.08; H, 7.58; N, 15.49. Found: C, 69, 07; H, 7.22; N, 15.26.

5.12. Ethyl 2-acetyl-4-oxo-4-[(4-substituted)phenyl]butanoate: general method

According to literature,[22,23] ethyl acetoacetate (1.56 g, 12 mmol) was added dropwise to a stirred suspension of sodium ethoxide (680 mg, 10 mmol) in 15 ml of anhydrous ethyl ether at rt under N_2. After 10 min a solution of phenacylbromide or 4-chlorophenacylbromide (10 mmol) in 26 ml of anhydrous ethyl ether was added dropwise, and the resulting mixture was heated at reflux for 2 h.

After cooling, the NaBr was filtered, washed twice with dry ether, and the joined organic phases were evaporated to give a product further purified by CC (silica gel; elution conditions as indicated for each compound).

5.12.1. Ethyl 2-acetyl-4-oxo-4-phenylbutanoate[22,23]

CC (cyclohexane/CH_2Cl_2; from 60:40 to 40:60). Yield: 93%. Oil. ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 7.43 Hz, 2H); 7.58 (t, J = 7.43 Hz, 1H); 7.48 (t, J = 7.43 Hz, 2H); 4.25–4.18 (m, 3H); 3.67 (dd, J = 18.43, 8.25 Hz, 1H); 3.43 (dd, J = 18.43, 8.25 Hz, 1H); 2.43 (s, 3H); 1.30 (t, J = 6.82 Hz, 3H).

5.12.2. Ethyl 2-acetyl-4-(4-chlorophenyl)-4-oxobutanoate[23]

CC (cyclohexane/AcOEt; 60:40); resulting solid rinsed with petroleum ether/dry ethyl 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). HRMS (ESI) m/z calcd for $C_{14}H_{15}ClO_4Na$ $[M+Na]^+$: 305.05511; found: 305.05570.

5.13. Ethyl 1-[(7-chloroquinolin-4-yl)amino]-2-methyl-5-[(4-substituted)phenyl]-1*H*-pyrrole-3-carboxylate (18 and 19): general method

The above diketoester (6.2 mmol) was added to a stirred solution of 7-chloro-4-hydrazinoquinoline (1.2 g, 6.2 mmol) in 7 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 and cyclohexane as indicated for each compound).

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

CC (AcOEt/cyclohexane; 40:60); solid rinsed with a mixture of ethyl ether–petroleum ether. Yield: 79%. Mp 165–168 °C. ^{1}H NMR (DMSO-d_6): δ 11.55 (s, 1/2H); 10.83 (s, 1/2H); 8.41 (s, 1H); 8.30 (s, 1/2H); 7.90 (s, 1/2H); 7.75–7.00 (m, 7H); 6.75 (s, 1H); 5.65 (s, 1/2H); 5.20 (s, 1/2H); 4.20 (q, J = 6.87 Hz, 2H); 2.30 (s, 3H); 1.30 (t, J = 6.87 Hz, 3H). HRMS (ESI) m/z calcd for $C_{23}H_{21}ClN_3O_2$ $[M+H]^+$: 406.13168; found: 406.13256.

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

CC (AcOEt/cyclohexane; 30:70); solid rinsed with ethyl ether. Yield 74%. Mp 210–212 °C. ^{1}H NMR (DMSO-d_6): δ 11.55 (s, 1/2H); 10.83 (s, 1/2H); 8.41 (s, 1H); 8.30 (s, 1/2H); 7.90 (s, 1/2H); 7.70–7.10 (m, 6H); 6.75 (s, 1H); 5.65 (s, 1/2H); 5.18 (s, 1/2H); 4.20 (q, J = 6.82 Hz, 2H); 2.30 (s, 3H); 1.30 (t, J = 6.82 Hz, 3H). HRMS (ESI) m/z calcd for $C_{23}H_{20}Cl_2N_3O_2$ $[M+H]^+$: 440.09271; found: 440.09350.

5.14. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-[(4-substituted)phenyl]-1*H*-pyrrole-3-(N-substituted)carboxamide (20a, b, f, g, 21a, and b): general method

A 2 M solution of $Al(CH_3)_3$ in toluene (0.625 ml, 1.25 mmol) was added dropwise to an ice-cooled stirred solution of the proper amine (1.25 mmol) in 10 ml of anhydrous toluene. The resulting mixture was stirred under N_2 at rt for 40 min, then **18** or **19** (1 mmol) was added. The solution was heated at reflux for 24 h, cooled and then treated with 2 N NaOH solution. The precipitate was filtered and washed four times with AcOEt, the joined organic layers were 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).

5.14.1. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-phenyl-1*H*-pyrrole-3-(*N*,*N*-diethyl)carboxamide (20a)

CC (CH_2Cl_2/MeOH; 95:5); solid rinsed with a mixture of ethyl ether/ethanol (95:5). Yield: 51%. Mp 252.7–254.3 °C. ^{1}H NMR (DMSO-d_6): δ 10.83 (br s, 1H); 8.41 (s, 1H); 7.90 (s, 1H); 7.70–7.00 (m, 7H); 6.50 (s, 1H); 5.66 (s, 1H); 3.41 (q, J = 6.82 Hz, 4H); 2.03 (s, 3H); 1.10 (t, J = 6.82 Hz, 6H). HRMS (ESI) m/z calcd for $C_{25}H_{26}ClN_4O$ $[M+H]^+$: 433.17897; found: 433.18035.

5.14.2. [1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-phenyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (20b)

During the reaction, a suspension was formed and a first portion of **20b** was collected and joined to the product obtained at the end of the procedure. CC (CH_2Cl_2/MeOH; 98:2). Yield: 86%. Mp 306.1–308.5 °C. ^{1}H NMR (DMSO-d_6): δ 10.80 (br s, 1H); 8.30 (s, 1H); 7.90 (s, 1H); 7.70–7.00 (m, 7H); 6.70 (s, 1H); 5.66 (s, 1H); 3.65 (s, 2H); 3.41 (s, 2H); 2.18 (s, 3H); 1.85 (s, 4H). HRMS (ESI) m/z calcd for $C_{25}H_{24}ClN_4O$ $[M+H]^+$: 431.16332; found: 431.16457.

5.14.3. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-phenyl-1*H*-pyrrole-3-*N*-[((1*S*,9a*R*)-octahydro-2H-quinolizin-1-yl)methyl]-carboxamide (20f)

During the reaction, a suspension was formed and compound **20f** was filtered and washed twice with toluene. This product was used directly in the next step without any purification. Yield: 88%. Mp 291.5–293.0 °C. ^{1}H NMR (DMSO-d_6): δ 10.75 (br s, 1H); 8.35 (s 1H); 8.10 (s, 1H); 7.90–7.65 (m, 2H); 7.65–7.00 (m, 6H); 6.90 (s, 1H); 5.50 (s, 1H); 3.35 (s, 2H); 2.85–2.60 (m, 2H); 2.30 (s, 3H); 2.00–1.10 (m, 14H). HRMS (ESI) m/z calcd for $C_{31}H_{35}ClN_5O$ $[M+H]^+$: 528.25246; found: 528.25451.

5.14.4. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-phenyl-1*H*-pyrrole-3-*N*-[2-((1*S*,9a*R*)-octahydro-2*H*-quinolizin-1-yl)ethyl]-carboxamide (20g)

CC (CH_2Cl_2/MeOH; from 94:6 to 90:10); solid rinsed with CH_2Cl_2/ethyl ether (30:70). Yield: 39%. Mp 197.6–200.5 °C. ^{1}H NMR (DMSO-d_6): δ 10.77 (br s, 1H); 8.40 (s, 1H); 8.30 (s, 1H); 7.90 (s, 2H); 7.70–7.00 (m, 6H); 6.90 (s, 1H); 5.66 (s, 1H); 3.30–3.00 (m, 2H); 2.80–2.58 (m, 2H); 2.25 (s, 3H); 2.10–1.10 (m, 16H). HRMS (ESI) m/z calcd for $C_{32}H_{37}ClN_5O$ $[M+H]^+$: 542.26811; found: 542.26899.

5.14.5. 5-(4-Chlorophenyl)-1-[(7-chloroquinolin-4-yl)amino]-2-methyl-1*H*-pyrrole-3-(*N*,*N*-diethyl)carboxamide (21a)

CC (CH_2Cl_2/MeOH; 99:1); solid rinsed with ethyl ether. Yield: 61%. Mp 261.5–263.8 °C. ^{1}H NMR (DMSO-d_6): δ 10.75 (br s, 1H); 8.45-8.15 (m, 2H); 7.90 (s, 1H); 7.65-7.35 (m, 3H); 7.21 (d, J = 8.25 Hz, 2H); 6.48 (s, 1H); 5.61 (s, 1H); 3.45 (q, J = 6.90 Hz, 4H); 2.05 (s, 3H); 1.12 (t, J = 6.90 Hz, 6H). HRMS (ESI) m/z calcd for $C_{25}H_{25}Cl_2N_4O$ $[M+H]^+$: 467.13999; found: 467.14081.

5.14.6. [5-(4-Chlorophenyl)-1-[(7-chloroquinolin-4-yl)amino]-2-methyl-1*H*-pyrrol-3-yl](pyrrolidin-1-yl)methanone (21b)

During the reaction, a suspension was formed and a first portion of **21b** was collected and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 98:2). Yield: 92%. Mp 324.5–326.5 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 11.00 (br s, 1H); 8.45–8.15 (m, 2H); 7.75 (s, 1H); 7.65–7.40 (m, 3H); 7.30 (d, J = 8.25 Hz, 2H); 6.75 (s, 1H); 5.50 (s, 1H); 3.70 (br s, 2H); 3.40 (br s, 2H); 2.15 (s, 3H); 1.81 (s, 4H). HRMS (ESI) m/z calcd for $C_{25}H_{23}Cl_2N_4O$ $[M+H]^+$: 465.12434; found: 465.12561.

5.15. 7-Chloro-4-[*N*-[2-methyl-5-phenyl-3-[(N-substituted-amino)-methyl]-1*H*-pyrrol-1-yl]-amino]quinoline (9a, b, f, and g): general method

Each amido compound **20** (1 mmol) was suspended in 43 ml of anhydrous ethyl ether under N_2 and $LiAlH_4$ (296 mg, 7.8 mmol) was carefully added; the mixture was heated at reflux and stirred for 4 h. After cooling, 1 N NaOH was added carefully and the suspension filtrated; alumina was washed twice with ethyl ether and the organic phase was dried (Na_2SO_4) and evaporated. The crude solid was purified by CC (silica gel; CH_2Cl_2–MeOH with or without concd NH_3 as indicated for each compound).

5.15.1. 7-Chloro-4-[*N*-[3-[(diethylamino)methyl]-2-methyl-5-phenyl-1*H*-pyrrol-1-yl]amino]quinoline (9a)

CC (CH_2Cl_2/MeOH; 95:5); solid rinsed with ethyl ether/petroleum ether (40:60). Yield: 66%. Mp 156.5–160 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 10.63 (s, 1H); 8.41 (d, J = 5.22 Hz, 1H); 8.30 (d, J = 9.08 Hz, 1H); 7.90 (s, 1H); 7.60 (d, J = 8.80 Hz, 1H); 7.50 (d, J = 7.43 Hz, 2H); 7.19 (t, J = 7.43 Hz, 2H); 7.08 (d, J = 7.30 Hz, 1H); 6.40 (s, 1H); 5.60 (d, J = 5.23 Hz, 1H); 3.45 (s, 2H); 2.01 (s, 3H); 1.02 (t, J = 6.87 Hz, 6H); four protons are missing probably because covered by the DMSO signal. HRMS (ESI) m/z calcd for $C_{25}H_{28}Cl_1N_4$ $[M+H]^+$: 419.19970; found: 419.19963.

5.15.2. 7-Chloro-4-[*N*-[2-methyl-5-phenyl-3-[(pyrrolidin-1-yl)methyl]-1*H*-pyrrol-1-yl]amino]quinoline (9b)

CC (CH_2Cl_2/MeOH; 96:4); solid washed with ethyl ether. Yield: 51%. Mp 172.5–176 °C. ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 1H); 8.40 (d, J = 5.22 Hz, 1H); 8.30 (d, J = 9.08 Hz, 1H); 7.90 (s, 1H); 7.60 (d, J = 8.80 Hz, 1H, CH); 7.50 (d, J = 7.43 Hz, 2H); 7.20 (t, J = 7.43 Hz, 2H); 7.09 (d, J = 7.30 Hz, 1H); 6.41 (s, 1H); 5.60 (d, J = 5.23 Hz, 1H); 3.55 (s, 2H); 2.65–2.50 (m, 4H); 2.02 (s, 3H); 1.85–1.55 (m, 4H). HRMS (ESI) m/z calcd for $C_{25}H_{26}ClN_4$ $[M+H]^+$: 417.18405; found: 417.18410.

5.15.3. 7-Chloro-4-[*N*-[2-methyl-3-[[((1S,9aR)-octahydro-2*H*-quinolizin-1-yl)methyl]aminomethyl]-5-phenyl-1*H*-pyrrol-1-yl]amino]quinoline (9f)

CC repeated twice (CH_2Cl_2/MeOH/concd NH_3; from 95:4.5:05 to 94:5.4:0.6). Solid rinsed with petroleum ether. Yield: 32%. Mp 93.5 °C (dec.). ^{1}H NMR ($CDCl_3$): δ 8.50 (s, 1H); 8.15–7.90 (m, 1H); 7.75–7.60 (m, 1H); 7.60–7.30 (m, 3H); 7.22–7.00 (m, 3H); 6.39 (s, 1H); 6.05 (s, 1H); 3.70 (s, 2H); 3.00–2.70 (m, 4H); 2.10 (s, 3H); 1.95–1.10 (m, 14H). HRMS (ESI) m/z calcd for $C_{31}H_{37}Cl_1N_5$ $[M+H]^+$: 514.27320; found: 514.27553. The free base was converted to trihydrochloride (mp 220–230 °C dec.), for biological tests.

5.15.4. 7-Chloro-4-[*N*-[2-methyl-3-[[2-((1S,9aR)-octahydro-2*H*-quinolizin-1-yl)ethyl]aminomethyl]-5-phenyl-1*H*-pyrrol-1-yl]amino]-quinoline (9g)

CC (CH_2Cl_2-MeOH; 93:7); solid washed with petroleum ether. Yield: 23%. Mp 104–107 °C (dec). ^{1}H NMR ($CDCl_3$): δ 8.60–8.10 (m, 2H); 7.90 (s, 1H); 7.70–7.30 (m, 3H); 7.20–6.90 (m, 3H); 6.55 (s, 1H); 5.75 (s, 1H); 3.90–3.50 (m, 2H); 3.05–2.50 (m, 4H); 2.50–1.90 (m, 5H); 1.90–0.95 (m, 14H). HRMS (ESI) m/z calcd for $C_{32}H_{39}ClN_5$ $[M+H]^+$: 528.28885; found: 528.28896.

5.16. 7-Chloro-4-[*N*-[3-[(diethylamino)methyl]-2-methyl-5-phenyl-1*H*-pyrrol-1-yl]amino]quinoline (9a): alternative method

Diethylamine (0.16 ml, 1.5 mmol), aq formaldehyde (37%, 0.13 ml, 1.5 mmol) were added at 0.5 ml of glacial acetic acid in an ice-cooled flask. The solution was stirred for 2–3 min and then poured into a flask containing **17** (501 mg, 1.5 mmol). The mixture was stirred at rt under N_2 for 1.5 h, water (15 ml) was added, and the solution alkalized with 2 N NaOH. The suspension was extracted with CH_2Cl_2 and the collected organic phases were dried (Na_2SO_4) and evaporated to give a crude product from which pure **9a** was obtained by three successive CC (silica, CH_2Cl_2 containing 3–7% MeOH). The joint solids containing the expected compound were washed with petroleum ether. Yield: 5%.

5.17. 7-Chloro-4-[*N*-[5-(4-chlorophenyl)-3-[(substitutedamino)-methyl]-2-methyl-1*H*-pyrrol-1-yl]amino]quinoline (10a and b): general method

To a stirred suspension of amide **21** (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 30% 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; CH_2Cl_2/MeOH, 94:6).

5.17.1. 7-Chloro-4-[*N*-[5-(4-chlorophenyl)-3-[(diethylamino)-methyl]-2-methyl-1*H*-pyrrol-1-yl]amino]quinoline (10a)

Solid rinsed with ethyl ether. Yield: 61%. Mp 215.6–220 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 10.60 (s, 1H); 8.39 (d, J = 5.22 Hz, 1H); 8.30 (d, J = 9.08 Hz, 1H); 7.90 (s, 1H); 7.61 (d, J = 8.80 Hz, 1H); 7.51 (d, J = 8.25 Hz, 2H); 7.21 (d, J = 8.25 Hz, 2H); 6.42 (s, 1H); 5.77 (d, J = 5.22 Hz, 1H); 3.55 (s, 2H); 2.10 (s, 3H); 1.15 (t, J = 6.87 Hz, 6H); four protons are missing probably because covered by the DMSO signal. HRMS (ESI) m/z calcd for $C_{25}H_{27}$ Cl_2N_4 $[M+H]^+$: 453.16073; found: 453.16154. The free base was converted to dihydrochloride (mp 185.5–191.8 dec.) for biological tests.

5.17.2. 7-Chloro-4-[*N*-[5-(4-chlorophenyl)-2-methyl-3-[(pyrrolidin-1-yl)methyl]-1*H*-pyrrol-1-yl]amino]quinoline (10b)

Solid rinsed with ethyl ether. Yield: 41%. Mp 179–182.3 °C. ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 1H); 8.40 (d, J = 5.22 Hz, 1H); 8.30 (d, J = 9.08 Hz, 1H); 7.90 (s, 1H); 7.65 (d, J = 8.80 Hz, 1H); 7.52 (d, J = 8.25 Hz, 2H); 7.25 (d, J = 8.25 Hz, 2H); 6.45 (s, 1H); 5.60 (d, J = 5.22 Hz, 1H); 3.45 (s, 2H); 3.30 (s, 2H); 2.00 (s, 3H); 1.70 (s, 4H); four protons are missing probably because covered by the DMSO signal. HRMS (ESI) m/z calcd for $C_{25}H_{25}Cl_2N_4$ $[M+H]^+$: 451.14508; found: 451.14505. The free base was converted to dihydrochloride (mp 207.5–211.7 °C dec.) for biological tests.

5.18. N-*tert*-Butyl-2-acetyl-4-oxo-4-phenylbutanamide (22)

A solution of N-*tert*-butyl-3-oxobutanamide (500 mg, 3.8 mmol)[25] in 3 ml of anhydrous ethyl ether was added dropwise to a stirred suspension of sodium ethoxide (216 mg, 3.18 mmol) in 14 ml of the same solvent at rt under N_2. After 10 min a solution of phenacylbromide (633 mg, 3.18 mmol) in 6 ml of dry ether was added dropwise and the resulting mixture was refluxed for 2 h.

After cooling, NaBr was filtered, washed twice with dry ether, and the organic phase was evaporated to give a crude product that was purified by CC (silica gel; cyclohexane/AcOEt, 85:15). Yield: 74%. Mp 111.6–113.7 °C. ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 7.43 Hz, 2H); 7.59 (t, J = 7.43 Hz, 1H); 7.47 (t, J = 7.43 Hz, 2H); 6.00 (s, 1H); 3.85 (t, J = 6.50 Hz, 1H); 3.67 (dd, J = 18.42, 6.50 Hz, 1H); 3.50 (dd, J = 18.42, 6.50 Hz, 1H); 2.35 (s, 3H); 1.35 (s, 9H). HRMS (ESI) m/z calcd for $C_{16}H_{21}NO_3Na$ $[M+Na]^+$: 298.14136; found: 298.14112.

5.19. 1-[(7-Chloroquinolin-4-yl)amino]-2-methyl-5-phenyl-1*H*-pyrrole-3-(*N*-*tert*-butyl)carboxamide (20c)

Compound **22** (500 mg, 1.82 mmol) was added to a stirred solution of 7-chloro-4-hydrazinoquinoline (352 mg, 1.82 mmol) in 3 ml of glacial acetic acid. The mixture was stirred and refluxed under N_2 for 2 h to give a violet solution, which, after cooling, was alkalinized with cold 2 N NaOH and extracted three times with CH_2Cl_2. The extract was dried (Na_2SO_4) and the solvent removed, leaving a solid that was purified by CC (silica gel; ethyl acetate/cyclohexane; 20:80). The product was rinsed with ethyl ether/ethanol (85:15). Yield: 80%. Mp 266.3–269.1 °C. ^{1}H NMR (DMSO-d_6): δ 10.72 (s, 1H); 8.52–8.21 (m, 2H); 7.90 (s, 1H); 7.70–7.10 (m, 7H);

7.02 (s, 1H); 5.60 (s, 1H); 2.30 (s, 3H); 1.37 (s, 9H). HRMS (ESI) *m*/*z* calcd for $C_{25}H_{26}ClN_4O$ $[M+H]^+$: 433.17897; found: 433.18007.

5.20. 4-[N-[3-[(*tert*-Butylamino)methyl]-2-methyl-5-phenyl-1*H*-pyrrol-1-yl]amino]-7-chloroquinoline (9c)

N-*tert*-Butyl-1-(7-chloroquinolin-4-ylamino)-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxamide (**8a**, 550 mg, 1.27 mmol) was suspended in 70 ml of anhydrous ethyl ether under N_2 and $LiAlH_4$ (550 mg, 14.5 mmol) was carefully added; then the mixture was refluxed and stirred for 5 h. After cooling, 1 N NaOH was added; the filtrated alumina was washed twice with ethyl ether and the organic layers were dried (Na_2SO_4) and evaporated. The crude was purified by CC (silica gel; CH_2Cl_2/MeOH, 95:5) to afford compound **2c**, which was rinsed with ethyl ether. Yield: 28%. Mp > 245 °C (dec.). ^1H NMR (DMSO-d_6): δ 10.50 (s, 1H); 8.37 (d, J = 5.21, 1H); 8.30 (d, J = 9.08, 1H); 7.85 (s, 1H); 7.70 (s, 1H); 7.61–7.42 (m, 3H); 7.18 (t, J = 7.43, 1H); 7.05 (d, J = 7.30, 1H); 6.41 (s, 1H); 5.61 (d, J = 5.21, 1H); 3.55 (s, 2H); 2.01 (s, 3H); 1.10 (s, 9H). HRMS (ESI) *m*/*z* calcd for $C_{25}H_{28}ClN_4$ $[M+H]^+$: 419.19970; found: 419.20173.

5.21. Parasite cultures and drug susceptibility assay

Plasmodium falciparum cultures were carried out according to Trager and Jensen with slight modifications.[32] 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 10% heat inactivated A-positive human plasma, 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 (OD_{650}) 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.[30] 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.[11]

5.22. Cell cytotoxicity assays

A long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV 40 large T antigen[33] was kindly provided by Dr. Francisco J. Candal, Center for Disease Control, Atlanta, GA, USA. Cells were 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 μg/ml of streptomycin, and 20 mM Hepes buffer (EuroClone). Unless stated otherwise, all reagents were from Sigma Italia, Milan, Italy. 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. For the cytotoxicity assays, cells were treated with serial dilutions of test compounds and cell proliferation evaluated using the MTT assay already described.[31] 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.

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