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4-Aminoquinoline quinolizidinyl- and quinolizidinylalkyl-derivatives with antimalarial activity

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Abstract—A set of quinolizidinyl and quinolizidinylalkyl derivatives of 4-amino-7-chloroquinoline and of 9-amino-6-chloro-2-methoxyacridine were prepared and tested in vitro against CQ-sensitive (D-10) and CQ-resistant (W-2) strains of *Plasmodium falciparum*. All compounds but one exerted significant antimalarial activity. Some of the quinolizidine derivatives were from 5 to 10 times more active than chloroquine on the CQ-resistant strain. No toxicity against mammalian cells was observed.

1. Introduction

In tropical and subtropical regions, malaria represents a serious health problem affecting 400–500 million people annually and leading to approximately 2.5 million deaths, mainly in sub-Saharan Africa.[1]

The most deadly species is *Plasmodium falciparum*, whose resistance to common antimalarials, such as chloroquine or antifolates, is increasing steadily worldwide.[2]

At present, the most promising and, so far, successful strategy in fighting malaria is a combination chemotherapy, in which an artemisinin derivative is used together with a conventional antimalarial to improve efficacy and delay onset of resistance.[3–5] Nevertheless, novel, effective, safe and inexpensive antimalarial agents are urgently needed to treat malaria in developing countries. There is also a need for new drugs that do not share the same mechanisms of resistance with those that are failing today. The quinoline type compounds continue to attract interest because their mechanisms of action and resistance are unrelated.

It is commonly accepted that chloroquine exerts its antimalarial activity by inhibiting hemozoin formation in the digestive vacuole of the parasite.[6–8] The exclusivity of this mechanism has been questioned; the inhibition of ferriprotoporphyrin IX degradation by glutathione-dependent redox processes could be an additional mode of action of chloroquine and its analogues.[7]

The cause of chloroquine resistance is not completely clear, but it is associated with an increased efflux from the parasite food vacuole and with mutations of Pfcrt genes of *P. falciparum*.[9,10]

Several chloroquine analogues and derivatives retain significant activity against chloroquine-resistant *P. falciparum* strains[11–14] and this has suggested that resistance could be compound-specific and not related to changes in the structure of the drug target.

Moreover, it has been observed that the dialkylaminoalkyl side chains introduced on 4-aminoquinoline may undergo oxidative dealkylation producing metabolites that display cross-resistance. On the contrary, chloroquine analogues containing the side chain basic nitrogen in a piperidine or pyrrolidine ring

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resulted in more metabolically stable compounds and exhibited a substantial increase in antimalarial activity against chloroquine-resistant strains.[15–17]

In the light of these observations, we studied the antimalarial activity of some 4-aminoquinoline derivatives, characterized by the presence of a bulky, strongly basic, and lipophilic bicyclic moiety as the quinolizidine ring (octahydro-2*H*-quinolizine), which is not supposed to be easily metabolized.

The quinolizidine ring may be directly linked to the 4-aminogroup (**1**–**4**) or through a chain of 1 to 3 carbon atoms (**5**–**10**). The same quinolizidinylalkyl moieties have also been introduced on the aminogroup of 9-amino-6-chloro-2-methoxyacridine (**12**–**15**) to produce analogues of quinacrine, an antimalarial agent largely used in the past, which is presently re-evaluated as inhibitor of trypanothione reductase of several trypanosome and leishmania species,[18] and as inhibitor of prion propagation in Creutzfeldt–Jacob disease.[19]

Finally, in continuing our investigations on metal-based antimicrobial[20,21] and antiproliferative agents,[22,23] and to verify whether the coordination with metal could enhance the activity of our 4-aminoquinoline derivatives, as observed for chloroquine,[24] complex (**11**) between gold and compound **5** was also prepared.

The structures of the presently investigated compounds are shown in Figure 1.

Six of these compounds have already been described by some of us and studied for different pharmacological activities: while compounds **5**–**7** and **12**–**14** were found inactive against P388 leukemia cells,[25] compounds **5** and **12**, when tested against 17 microbial species, showed good activity only against *Mycobacterium tuberculosis* (MIC = 5 and 10 μg/ml, respectively) and *Trichomonas vaginalis* (MIC = 50 and 12.5 μg/ml, respectively).[26] Russian authors described some antimalarial activity of compound **12**, a long time ago.[27]

All compounds were tested in vitro for activity against chloroquine-sensitive (CQ-S) and chloroquine-resistant (CQ-R) strains of *P. falciparum* and for cytotoxicity against mammalian cells; compounds with the highest in vitro activity are under scrutiny in vivo against *P. berghei* and *P. yoelii* in mice.

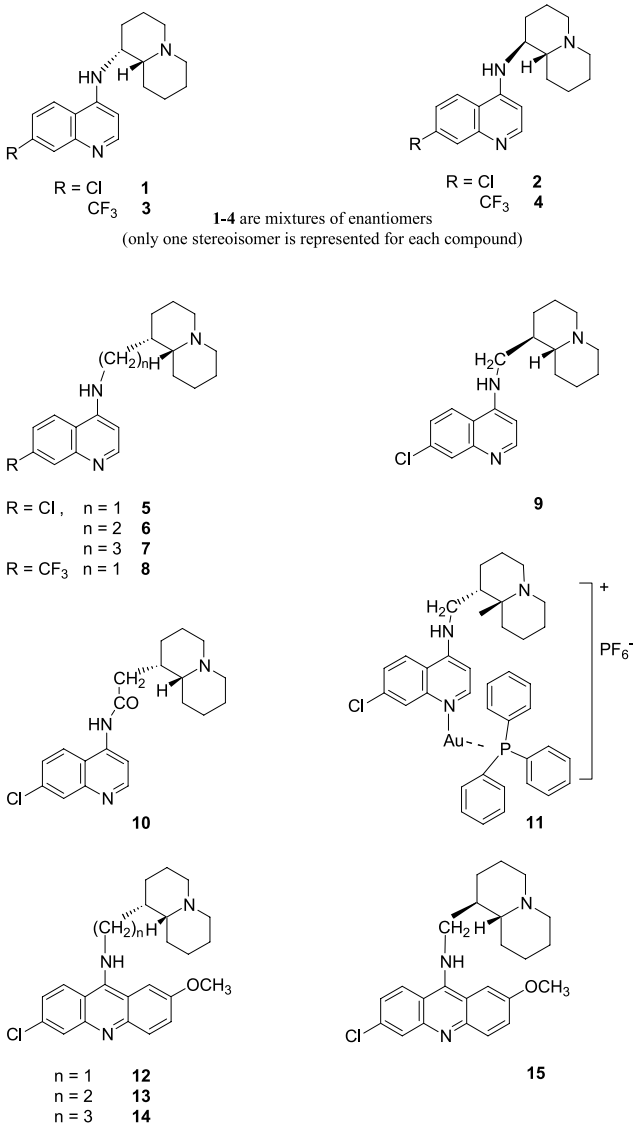
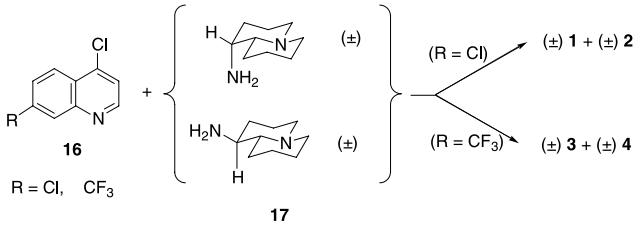


Figure 1. Structures of the investigated compounds (absolute configurations).



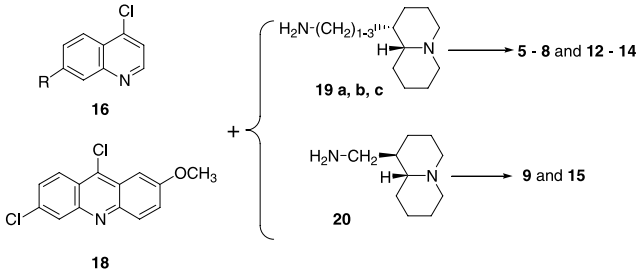
Scheme 1.

2. Chemistry

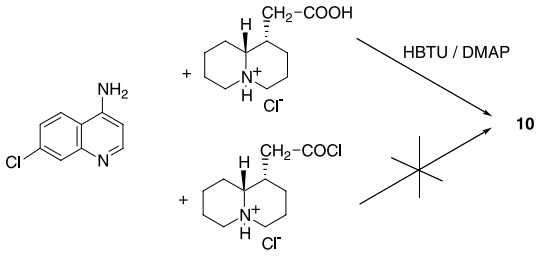
Compounds **1**–**4** were prepared by reacting in the presence of phenol the 4,7-dichloroquinoline or the 4-chloro-7-trifluoromethylquinoline with 1-aminoquinolizidine (octahydro-2*H*-quinolizin-1-amine, **17**), which was obtained as a diastereoisomeric mixture by the $LiAlH_4$ reduction of 1-oxoquinolizidine oxime[28,29] (Scheme 1).

Separation of diastereoisomers was delayed until after the conversion to quinoline derivatives, since the latter can be easily separated by chromatography (silica; CH_2Cl_2 containing 1–8% of MeOH, v/v).

Assignment of the structure was based on NMR spectra. When the aminogroup is axial, the hydrogen atom of position 1 (H-1) exhibits two trans axial–axial and one cis axial–equatorial couplings with coupling constants higher than those characterizing the epimer (two cis equatorial–axial and one trans equatorial–equatorial couplings).[30] Indeed, compounds **2** and **4** exhibited



Scheme 2.



Scheme 3.

multiplets within δ 3.40–3.60, while compounds **1** and **3** showed a pseudo doublet with δ 3.55–3.70.

Quinolizidinylalkyl derivatives **5**–**9** and **12**–**15** were prepared by reaction of the suitable quinolizidinylalkylamines **19a**–**c** (n = 1–3) and **20** with 4,7-dichloro or 4-chloro-7-trifluoromethylquinoline and 6,9-dichloro-2-methoxyacridine, respectively (Scheme 2).

The required amines were prepared following the methods previously described for lupinylamine (**19**, n = 1),[31] for 2-(quinolizidin-1′α-yl)ethylamine and 3-(quinolizidin-1′α-yl)propylamine (**19**, n = 2 and 3)[32] and for *epi*-lupinylamine **20.**[33]

The homolupinanoylamide **10** was obtained by condensation of 4-amino-7-chloroquinoline[34] with homolupinanoic acid hydrochloride[26,35] ((1*S*,9a*R*)-octahydro-2*H*-quinolizine-1-ethanoic acid) in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate (HBTU) and 4-dimethylaminopyridine (DMAP) (Scheme 3).

A preliminary attempt to prepare **10** by reacting 4-amino-7-chloroquinoline with the homolupinanoylchloride hydrochloride gave only very poor results.

Finally, the reaction of triphenylphosphine gold(I)chloride with compound **5** and potassium hexafluorophosphate in acetonitrile afforded the gold complex **11.**

Structures of final compounds were supported by elemental analyses and spectral data.

3. Results and discussion

All synthesized compounds 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). Cytotoxicity on murine cells WEHI, clone 13, was assayed using the MTT test.

Table 1 shows the IC_{50} (nM) against D-10 and W-2 strains of *P. falciparum* as well as the means of the ratios between the IC_{50} of chloroquine and that of each com-

Table 1. Antimalarial activity of compounds **1**–**15** tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *Plasmodium falciparum*

Compound	D-10 (CQ-S) IC_{50} (nM)[a]	No. of Exp.	Ratio IC_{50} CQ/comp[b]	W-2 (CQ-R) IC_{50} (nM)[a]	No. of Exp.	Ratio IC_{50} CQ/comp[b]	Ratio IC_{50} CQ-R /CQ-S[c]	WEHI 13[d] IC_{50} (nM)
1	24.73 ± 15.64	8	1.11	23.34 ± 8.10	8	9.42	0.94	15,647
2	24.20 ± 14.58	8	1.32	21.27 ± 7.65	8	9.69	0.88	18,104
3	102.46 ± 29.55	5	0.17	106.57 ± 14.38	4	2.12	1.04	n.t.
4	71.74 ± 23.78	5	0.23	73.83 ± 32.70	4	3.42	1.03	n.t.
5	26.36 ± 15.29	13	0.87	41.87 ± 19.86	12	6.93	1.59	9206
6	26.08 ± 11.07	3	1.03	181.57 ± 61.10	3	1.67	6.96	>14,539
7	45.96 ± 21.87	4	0.60	384.40 ± 136.75	2	0.88	8.36	10577.48
8	33.68 ± 11.33	6	0.45	31.90 ± 9.15	4	7.44	0.95	n.t.
9	31.34 ± 8.15	4	1.17	94.97 ± 31.03	3	4.22	3.03	n.t.
10	⩾5030.00	2	0.006	⩾3968.00	2	0.03	0.79	n.t.
11	28.44 ± 12.94	4	1.30	52.93 ± 30.23	4	10.07	1.86	n.t.
12	36.99 ± 21.60	7	0.93	80.35 ± 30.38	6	5.59	2.17	2475
13	30.78 ± 7.11	3	0.75	68.13 ± 29.92	3	5.13	2.21	792
14	43.45 ± 14.43	3	0.53	97.44 ± 6.88	4	3.16	2.24	1053
15	35.22 ± 14.63	4	1.02	95.37 ± 39.12	4	5.07	2.70	n.t.
CQ	24.68 ± 15.75	19	1.00	276.53 ± 149.35	18	1.00	11.20	>9692

n.t.: not tested.

[a] The results are expressed as IC_{50} ± SD of different experiments each performed in duplicate.

[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 murine cell line WEHI Clone 13 using the MTT assay.

pound against D-10 or W-2 strains calculated for each single experiment. 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).

With only one exception (compound **10**), all tested compounds exhibited a high degree of activity on the CQ-S, D-10 strain, with IC_{50} in the range from 24.2 to 102.4 nM. Chloroquine IC_{50} was 24.7 nM (range 15–38 nM); thus the tested compounds were from 0.17- to 1.32-fold as active as chloroquine.

Some of the new compounds also exhibited a strong activity against the CQ-R, W-2 strain, with IC_{50} values as low as 21–23 nM (**1** and **2**) compared to 276.5 nM of chloroquine (range 126–468 nM). Therefore, five compounds (**5**, **8**, **1**, **2**, and **11**) resulted from 6.9- to 10.1-fold more active than chloroquine and seven compounds were from 2.1 to 5.6 times more active than the reference drug. Only compounds **6** and **7** did not show significant differences of activity versus chloroquine on either strain.

The novel chloroquine analogues were either equitoxic or definitely less toxic than chloroquine on murine cells WEHI (clone 13), while the acridine derivatives **12**–**14** were more toxic than the reference drug as was already known for quinacrine.

A thorough comparison of our compounds with other chloroquine analogues is not easy, since different strains of CQ-R *P. falciparum* were used. Nevertheless, our best compounds (**5**, **8**, **1**, **2**, and **11**) appear to be at least as comparable and often more active than some recently described short side-chain analogues.[11,13,15]

Indeed, the best of the latter compounds (7-chloro-4-[*N*-(diethylaminoethyl)amino]quinoline) was 6.4- and 19.0-fold more active than chloroquine against the CQ-R K1[11] and Indochina I[13] strains, respectively, while our compounds were 6.9- to 10.1-fold more active than chloroquine against the CQ-R, W-2 strain.

With regards to the structure–activity relationships, it has been observed that the structure modifications did not influence the activities on CQ-S and CQ-R strains of *P. falciparum* in the same manner.

Major comments are as follows.

The activity was negatively influenced by the increasing number (n) of methylene groups between the aminoquinoline and the quinolizidine moieties. On comparing compound **1** ($n = 0$) and compound **7** ($n = 3$), the decrease of activity was modest in the case of CQ-S strain (**1**, $IC_{50} = 24.7$ nM; **7**, $IC_{50} = 46.0$ nM), but it became substantial for CQ-R strain (**1**, $IC_{50} = 23.3$ nM; **7**, $IC_{50} = 384.4$ nM).

Minor variations of activity with the increasing length of the methylene chain were also observed in the set of acridine derivatives (**12**–**14**) though the differences in IC_{50} were not significant. If one considers the ratios of compounds **12**–**14** with chloroquine, the trend toward less activity with increasing length of the methylene chain becomes more evident.

Comparing activities shown by the couples of epimeric compounds (**1** and **2**, **3** and **4**, **5** and **9**, **12** and **15**), we have observed that the shift of the amino- or the aminomethylene-group from α to β position at C(1) of the quinolizidine ring produced only very modest changes of activity on both CQ-S and CQ-R strains: an increased activity was shown by compound **4** compared to **3** on both *P. falciparum* strains, whereas a negative variation was seen for compound **9** only against the W-2 strain. None of these effects reached statistical significance.

The replacement of the chlorine atom on the quinoline ring with a trifluoromethyl group strongly reduced the activity on both CQ-S and CQ-R strains when the secondary aminogroup is directly linked to the quinolizidine ring as in compound **3** and **4**. A similar effect of the 7-substituent in other chloroquine analogs was already observed by De et al.[13] and Stocks et al.[15] on the activity against different CQ-S and CQ-R strains of *P. falciparum*, and by Kaschula et al.[8] against the same CQ-S, D-10 strain used in this paper. The latter authors correlated the decreased activity with the stronger electron-withdrawing character of the trifluoromethyl group, which reduced the basicity of both the basic nitrogen atoms, though its higher lipophilicity should improve the association with hematin and the inhibition of hemozoin formation.

On the contrary, the homologous fluorinated compound **8**, as compared to the corresponding chloro-derivative **5**, displayed only minor variations of activity against both the CQ-S and CQ-R strains. In the case of 4-[(*N*-*tert*-butylamino)ethyl]amino-7-trifluoromethylquinoline,[15] the positive effect of enhanced lipophilicity seems to overcome the negative effect of the reduced basicity on the activity against the CQ-R strain. Indeed, the degree of drug lipophilicity correlates with the ability of antimalarials to overcome CQ-resistance in vitro.[16,17]

According to Navarro et al.[24] the complexation of chloroquine with gold was able to overcome resistance. A similar complexation with gold of our compound **5**, which is itself very active on CQ-R strain, produced compound **11** that resulted 10 times more active than chloroquine on CQ-R, W-2 strain. Although the high cost restrains an interest in this compound, the study of other complexes of the new compounds with less expensive metals is surely warranted.

Finally, we have seen that the acylation of 4-amino-7-chloroquinoline with a homolupinanoyl residue practically abolished the activity. Indeed, compound **10** exhibited a IC_{50} that was >14 fold that of chloroquine versus CQ-R strain W-2 and >203-fold that versus the CQ-S strain D-10. Also in comparison to other quinolizidine derivatives, the amide **10** resulted 1 to 2 order of magnitude less active.

The possibility that the poor activity of amide **10** is due to hydrolysis under the test conditions should be ruled out, since compound **10** was unaffected by incubation for 72 h at 37 °C in PBS (phosphate buffered saline pH 7.3) as well as in 0.1 N HCl or 0.1 N NaOH solutions.

Alternatively, it is conceivable that such a loss of activity is related to the prevailing electron delocalization from the secondary aminic nitrogen atom to the carbonyl oxygen atom, instead to the heterocyclic nitrogen atom (Scheme 4).

The importance of resonance between the nitrogen atoms of the aminogroup and of the quinoline ring was underlined to explain the association of chloroquine with ferriprotoporphyrin IX (Fe(III)PPIX) and hence its antimalarial activity, though the question is far from being settled.[6]

The electrostatic hindrance to charge delocalization between the two nitrogen atoms was also sought to explain the strong decrease of antimalarial activity with the shortening of the aliphatic basic chain in a set of quinacrine analogues.[36]

4. Conclusions

All but one of the tested compounds were active against both CQ-S (D-10) and CQ-R (W-2) strains of *P. falciparum*, the best being from 6.9 to 10 times more active than chloroquine on W-2 strain. The same compounds were equitoxic or definitely less toxic than chloroquine on murine cell WEHI (clone 13). Therefore, the bulky basic head which characterizes the prepared compounds appears to be an interesting structural feature able to overcome the resistance mechanisms and deserves further investigation to obtain novel antimalarial agents.

In vivo studies in the rodent *P. berghei* malaria model are underway.

5. Experimental

5.1. General

All commercially available solvents and reagents were used without further purification, unless otherwise stated. CC = column chromatography. Mps: Büchi apparatus, uncorrected. IR spectra: Perkin-Elmer-Paragon-1000 Pc spectrophotometer; KBr pellets for solid and net for liquid; ν in cm^{-1}. ^1H NMR spectra: Varian Mercury 300VX spectrometer; $CDCl_3$ or DMSO-d_6 with Me_4Si as internal standard; δ in ppm, J in hertz. 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.

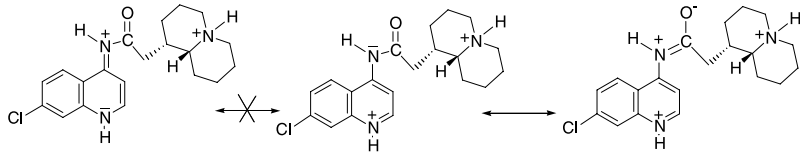
5.2. 4-[*N*-(Octahydro-2*H*-quinolizin-1α- and 1-β-yl)amino]-7-substituted-quinoline (1–4). General method

Octahydro-2*H*-quinolizin-1-ylamine (mixture of diastereoisomers;[29] 5 g, 10 mmol) was added to a solution of 10 mmol of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline in 6 g of phenol and was heated at 180 °C under N_2 for 4 h. After cooling, the mixture was treated with 2 M NaOH till strong alkalinity and extracted with Et_2O. The organic phase was shaken with 2 M NaOH, then with H_2O and extracted with 5% acetic acid. The acid solution was alkalinized with 2 M NH_3 and extracted with Et_2O. After drying, the solvent was removed and the residue was divided into its epimeric components by CC (silica; CH_2Cl_2 containing 1–8% of MeOH, v/v): (±)1α-substituted epimers (**1** and **3**) were eluted first (CH_2Cl_2 + 1–2% MeOH), followed by the (±)β-ones (**2** and **4**) (CH_2Cl_2 + 3–8% MeOH).

5.2.1. (±)7-Chloro-4-[*N*-(octahydro-2*H*-quinolizin-1α-yl)amino]quinoline (1). Yield 15%; mp 168–169 °C (Et_2O). ^1H NMR ($CDCl_3$): δ 1.00–2.30 (m, 13H, quinolizidine = QZ); 2.80–3.00 (m, 2H, H-α near N of QZ); 3.60 (d, J = 8.2 Hz, 1H, C(1)H of QZ, s after D_2O exchange); 5.95 (d, J = 8.2 Hz, 1H, NH, collapses with D_2O); 6.35 (d, J = 5.4 Hz, 1H, C(3)H of quinoline = QN); 7.40 (dd, J = 8.6, 1.4 Hz, 1H, C(6)H of QN); 7.80 (d, J = 8.4 Hz, 1H, C(5)H of QN); 7.95 (pseudo s, 1H, C(8)H of QN); 8.50 (d, J = 5.4 Hz, 1H, C(2)H of QN). Anal. Calcd for $C_{18}H_{22}ClN_3$: C, 68.43; H, 7.02; N, 13.36. Found: C, 68.60; H, 7.18; N, 13.41.

5.2.2. (±)7-Chloro-4-[*N*-(octahydro-2*H*-quinolizin-1β-yl)amino]quinoline (2). Yield 5%; mp 235–238 °C (abs EtOH). ^1H NMR ($CDCl_3$): δ 1.00–2.30 (m, 13H, QZ); 2.80–3.00 (m, 2H, H-α near N of QZ); 3.35–3.60 (m, 1H, C(1)H of QZ, m after D_2O exchange); 4.65 (d, J = 8.0 Hz, 1H, NH, collapses with D_2O); 6.50 (d, J = 5.4 Hz, 1H, C(3)H of QN); 7.35 (dd, J = 8.2, 1.4 Hz, 1H, C(6)H of QN); 7.65 (d, J = 8.8 Hz, 1H, C(5)H of QN); 7.95 (pseudo s, 1H, C(8)H of QN); 8.50 (d, J = 5.4 Hz, 1H, C(2)H of QN). Anal. Calcd for $C_{18}H_{22}ClN_3$: C, 68.43; H, 7.02; N, 13.36. Found: C, 68.25; H, 6.94; N, 13.28.

5.2.3. (±)4-[*N*-(Octahydro-2*H*-quinolizin-1α-yl)amino]-7-trifluoromethylquinoline (3). Yield 15%; mp 132.5–135 °C (Et_2O). ^1H NMR ($CDCl_3$): δ 1.00–2.30 (m,



Scheme 4.

13H, QZ); 2.70–3.00 (m, 2H, H-α near N of QZ); 3.55 (d, J = 8.2 Hz, 1H, C(1)H of QZ, s after D_2O exchange); 5.98 (d, J = 8.2 Hz, 1H, NH, collapses with D_2O); 6.35 (d, J = 5.4 Hz, 1H, C(3)H of QN); 7.53 (dd, J = 8.6, 1.4 Hz, 1H, C(6)H of QN); 7.90 (d, J = 8.4 Hz, 1H, C(5)H of QN); 8.19 (pseudo s, 1H, C(8)H of QN); 8.50 (d, J = 5.4 Hz, 1H, C(2)H of QN). Anal. Calcd for $C_{19}H_{22}F_3N_3$: C, 65.31; H, 6.35; N, 12.03. Found: C, 64.97; H, 6.60; N, 11.97.

5.2.4. (±)4-[*N*-(Octahydro-2*H*-quinolizin-1β-yl)amino]-7-trifluoromethylquinoline (4). Yield 14.7%; mp 201–204 °C (Et_2O). ^1H NMR ($CDCl_3$): δ 1.00–2.30 (m, 13H, QZ); 2.70–3.00 (m, 2H, H-α near N of QZ); 3.30–3.55 (m, 1H, C(1)H of QZ, m after D_2O exchange); 4.67 (d, J = 8.0 Hz, 1H, NH, collapses with D_2O); 6.50 (d, J = 5.4 Hz, 1H, C(3)H of QN); 7.51 (dd, J = 8.2, 1.4 Hz, 1H, C(6)H of QN); 7.75 (d, J = 8.8 Hz, 1H, C(5)H of QN); 8.19 (pseudo s, 1H, C(8)H of QN); 8.54 (d, J = 5.4 Hz, 1H, C(2)H of QN). Anal. Calcd for $C_{19}H_{22}F_3N_3$: C, 65.31; H, 6.35; N, 12.63. Found: C, 65.41; H, 6.55; N, 11.96.

5.3. 7-Chloro-4-{*N*-[ω-(octahydro-2*H*-quinolizin-1α-yl)-alkyl]amino}-quinolines (5–7)

Compounds **5**–**7** were prepared as described by Boido Canu et al.[25]

5.4. 4-{*N*-[(Octahydro-2*H*-quinolizin-1α-yl)methyl]amino}-7-trifluoromethyl-quinoline (8)

A mixture of (1*S*,9a*R*)-octahydro-2*H*-quinolizine-1-methanamine[31] (**19**, n = 1; 0.530 g, 3.15 mmol), 4-chloro-7-trifluoromethylquinoline (0.725 g, 3.15 mmol) and phenol (2 g) was heated for 3 h at 180 °C under N_2. After cooling, the mixture was treated with 2M NaOH till strong alkalinity and extracted with Et_2O. The organic solution was shaken with 2 M NaOH, then with H_2O and extracted with 5% acetic acid. The acid solution was alkalinized with 2 M NH_3 and extracted with Et_2O. After evaporation, the residue (1.05 g) was purified by CC (silica, CH_2Cl_2 containing 2% MeOH). Compound **8**: 0.97 g, 88.2%; mp 84.5–86.5 °C (Et_2O–petroleum ether). ^1H NMR ($CDCl_3$): δ 1.0–2.3 (*m*, 14H, QZ); 2.90–3.10 (m, 2H, H-α near N of QZ); 3.26 (dd, J = 12.8, 6.2 Hz, 1H of CH_2–NH; d, J = 11.8 Hz after D_2O exchange); 3.52 (dd, J = 12.8, 6.2 Hz, 1H of CH_2–NH); 6.25 (d, J = 5.4 Hz, 1H, C(3)H of QN); 7.50 (dd, J = 8.8, 1.8 Hz, 1H, C(6)H of QN); 7.78 (d, J = 8.8 Hz, 1H, C(9)H of QN); 8.16 (s, 1H, C(8)H of QN); 8.50 (d, J = 5.4 Hz, 1H, C(2)H of QN); 8.70–8.90 (broad s, 1H, NH collapses with D_2O). Anal. Calcd for $C_{20}H_{24}F_3N_3$: C, 66.10; H, 6.66; N, 11.56. Found: C, 66.24; H, 6.29; N, 11.26.

5.5. 7-Chloro-4-{*N*-[(octahydro-2*H*-quinolizin-1β-yl)methyl]amino}-quinoline (9)

A mixture of (1*R*,9a*R*)-octahydro-2*H*-quinolizine-1-methanamine[33] (**20**, 0.55 g, 3.27 mmol), 4,7-dichloroquinoline (0.64 g, 3.27 mmol) and phenol (2 g) was heated for 4 h at 180 °C under N_2. After cooling, the mixture was treated with 2 M NaOH and the precipitated was filtered and washed with H_2O. The joined aq solution was extracted with Et_2O. The organic phase was shaken with 2 M NaOH, then with H_2O and extracted with dilute acetic acid. The acid solution was alkalinized with 2 M NH_3 and extracted with ether. After evaporation, the residue was joined with the filtered solid and crystallized from abs EtOH/Et_2O. Compound **9**: 0.51 g, 47%; mp 118–125 °C. ^1H NMR (DMSO-d_6): δ 0.9–2.1 (m, 14H, QZ); 2.60–2.80 (m, 2H, H-α near N of QZ); 2.80–3.00 (m, 1H of CH_2–NH); 3.48 (d, J = 12.4 Hz, 1H of CH_2–NH); 6.40 (d, J = 5.4 Hz, 1H, C(3)H of QN); 7.15–7.25 (m, 1H, NH collapses with D_2O); 7.43 (d, J = 8.8 Hz, 1H, C(6)H of QN); 7.75 (s, 1H, C(8)H of QN); 8.28 (d, J = 8.8 Hz, 1H, C(5)H of QN); 8.35 (d, J = 5.4 Hz, 1H, C(2)H of QN). Anal. Calcd for $C_{19}H_{24}ClN_3 \cdot H_2O$: C, 65.59; H, 7.53; N, 12.08. Found: C, 65.78; H, 7.49; N, 12.04.

5.6. 7-Chloro-4-[*N*-(homolupinanoyl)amino]-quinoline (10)

A solution of 4-amino-7-chloroquinoline[34] (0.1 g, 0.56 mmol), homolupinanoic acid hydrochloride[26,35] (0.13 g, 0.56 mmol), DMAP (0.14 g, 1.12 mmol), HBTU (0.43 g, 1.12 mmol), triethylamine (0.14 mL) in DMF and CH_2Cl_2 (8+2 mL) was stirred at rt for 8 h. Solvents were distilled in vacuo; 0.5 M NaOH was added till pH 9–10 and the mixture extracted with CH_2Cl_2. After evaporation, the residue was firstly purified by CC (silica, CH_2Cl_2 containing 1–5% MeOH) to eliminate the unreacted aminoquinoline and DMAP, and then by preparative TLC (silica, CH_2Cl_2/MeOH/concd NH_3, 50:10:0.5, v/v). Compound **10**: 0.025 g, 12.5%; mp 192.5–193.5 (Et_2O). ^1H NMR ($CDCl_3$): δ 1.30–2.40 (m, 14H, QZ); 2.74 (dd,J = 15.0, 6.0 Hz, 1H of $COCH_2$); 2.80–2.95 (m, 3H, 1H of $COCH_2$ + 2H of QZ); 7.52 (dd,J = 8.6, 1.4 Hz, 1H C(6)H of QN); 7.75 (d,J = 8.8 Hz, 1H, C(5)H of QN); 8.10 (s, 1H, C(8)H of QN); 8.30 (d, J = 5.4 Hz, 1H, C(3)H of QN); 8.82 (m, 2H, C(2)H of QN + NH; partially collapses with D_2O). Anal. Calcd for $C_{20}H_{24}ClN_3O$: C, 67.12; H, 6.76; N, 11.74. Found: C, 67.30; H, 6.67; N, 11.79.

The title compound (9 mg, 0.025 mmol) was dissolved in 0.5 mL of DMSO and added to 9 mL of PBS (phosphate buffered saline pH 7.3). The solution was incubated at 37 °C for 3 days and afterward made alkaline and extracted with CH_2Cl_2 with a practically quantitative recovery of the amide (TLC, silica, CH_2Cl_2/MeOH/concd NH_3, 50/10/0.5, v/v). A similar experiment performed with 9 mL of 0.1 N HCl or 0.1 N NaOH solution gave an identical result.

5.7. [(Goldtriphenylphosphine)-(7-chloro-4-{*N*-[(octahydro-2*H*-quinolizin-1α-yl)methyl]amino}quinoline)] hexafluorophosphate (11)

Triphenylphosphinogold(I) chloride (0.20 g, 0.4 mmol) was dissolved in acetonitrile (20 mL) by mild heating under N_2, then potassium hexafluorophosphate (0.149 g, 0.8 mmol) was added and refluxing was continued for 30 min. Compound **5** (0.264 g, 0.8 mmol) was

added and the mixture was refluxed under N_2 for 48 h. After cooling, the precipitate was filtered and the solution was concentrated to small volume, Et_2O was added and after standing in freezer overnight, again filtered. The solution was finally evaporated to dryness and taken up in Et_2O that left **11** as a brownish powder (0.200 g, 53.5%). Anal. Calcd for $C_{37}H_{39}AuClF_6N_3P_2$: C, 47.58; H, 4.21; N, 4.50. Found: C, 51.49; H, 4.61; N, 4.69.

5.8. 6-Chloro-2-methoxy-9-{*N*-[ω-(octahydro-2*H*-quinolizin-1α-yl)alkyl]amino}-acridines (12–14)

Compounds **12**–**14** were prepared as described by Boido Canu et al.[25]

5.9. 6-Chloro-2-methoxy-9-{*N*-[(octahydro-2*H*-quinolizin-1β-yl)methyl]amino}acridine (15)

A mixture of (1*R*,9a*R*)-octahydro-2*H*-quinolizine-1-methanamine[33] (**20**, 0.336 g, 2 mmol), 6,9-dichloro-2-methoxyacridine (0.573 g, 2 mmol) and phenol (1.25 g) was heated for 3 h at 100 °C under N_2. After cooling, the mixture was treated with 2 M NaOH till strong alkalinity and extracted with Et_2O. The organic phase was washed with 2 M NaOH, then with H_2O and extracted with 5% acetic acid. The acid solution was alkalinized with 2 M NH_3 and extracted with Et_2O. After evaporation, the residue was purified by CC (silica, CH_2Cl_2 containing 2% MeOH). Compound **15**: 0.144 g, 18%; mp 92–96 °C (Et_2O). ^{1}H NMR (DMSO-d_6): δ 0.90–1.95 (m, 14H, QZ); 2.50–2.75 (m, 2 H, H-α near N of QZ); 3.40–3.50 (m, 1H, *CH*$_2$–NH); 3.90 (s, 3H, OCH_3); 4.05 (d, J = 12.7 Hz, 1H, *CH*$_2$–NH); 6.80 (s, 1H, NH collapses with D_2O); 7.30 (d, J = 9.2 Hz, 1H, Ar); 7.40 (d, J = 9.2 Hz, 1H, Ar); 7.60 (s, 1H, Ar); 7.80 (d, J = 9.2 Hz, 1H, Ar); 7.83 (s, 1H, Ar); 8.30 (d, J = 9.2 Hz, 1H, Ar). Anal. Calcd for $C_{24}H_{28}ClN_3O$: C, 70.35; H, 6.89; N, 10.26. Found: C, 69.94; H, 6.89; N, 10.18.

5.10. Parasite cultures and drug susceptibility assay

P. falciparum cultures were carried out according to Trager and Jensen's with slight modifications.[37] The CQ-sensitive, strain D-10 and the CQ-resistant, strain W-2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone, Celbio) ($NaHCO_3$ 24 mM) medium with the addition of 10% heat-inactivated A-positive human plasma, 20 mM Hepes, 2 mM glutammine. All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, 94% N_2. Compounds were dissolved in either water (chloroquine) 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 wells flat-bottom microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitemia 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.[38,39] 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.

5.11. Cell proliferation assay

The proliferation of the immortalized WEHI Clone 13 murine cell line was tested using the MTT assay already described.[40] 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 were 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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