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Synthesis and Antiplasmodial Activity of Novel Chloroquine Analogues with Bulky Basic Side Chains

Bruno Tasso,*[a] Federica Novelli,[a] Michele Tonelli,[a] Anna Barteselli,[b] Nicoletta Basilico,[c] Silvia Parapini,[d] Donatella Taramelli,[d] Anna Sparatore,[b] and Fabio Sparatore[a]

Chloroquine is commonly used in the treatment and prevention of malaria, but *Plasmodium falciparum*, the main species responsible for malaria-related deaths, has developed resistance against this drug. Twenty-seven novel chloroquine (CQ) analogues characterized by a side chain terminated with a bulky basic head group, i.e., octahydro-2*H*-quinolizine and 1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one, were synthesized and tested for activity against D-10 (CQ-susceptible) and W-2 (CQ-resistant) strains of *P. falciparum*. Most compounds were found to be active against both strains with nanomolar or sub-micromolar IC_{50} values. Eleven compounds were found to be 2.7- to 13.4-fold more potent than CQ against the W-2 strain; among them, four cytisine derivatives appear to be of particular interest, as they combine high potency with low cytotoxicity against two human cell lines (HMEC-1 and HepG2) along with easier synthetic accessibility. Replacement of the 4-NH group with a sulfur bridge maintained antiplasmodial activity at a lower level, but produced an improvement in the resistance factor. These compounds warrant further investigation as potential drugs for use in the fight against malaria.

Introduction

Recent estimates suggest that more than 198 million clinical cases of malaria occur every year worldwide, with 584 000 deaths in 2013. Most of the casualties are children living in sub-Saharan Africa or pregnant women.[1] *Plasmodium falciparum* (*Pf*), which is the main species responsible for the observed fatalities, has developed resistance to conventional antimalarials such as chloroquine (CQ) and antifolates. Resistance even to the artemisinin derivatives has been recently reported in southeast Asia.[2] To overcome the drug resistance issue, extensive modification of existing antimalarial chemotypes and the search for novel targets (and new chemotypes interacting with them) have been undertaken and are currently being pursued. Thus, many interesting compounds have been selected for use in monotherapy or, even better, in combination chemotherapies (particularly artemisinin-based combination therapies) able to improve efficacy and delay the onset of resistance. The different approaches are illustrated and discussed in some recent reviews.[3]

Particularly, the 4-aminoquinoline derivatives continue to attract interest because resistance appears to be compound specific and not related to changes in the structure of the drug targets.[4] Indeed, even close analogues of CQ and amodiaquine retain potent activity against CQ-resistant (CQ-R) strains of *P. falciparum*, such as short side-chain compounds **A**–**C**[5] and metabolically stable isoquine (**D**), *tert*-butylisoquine (**E**), and 4′-fluoro-*N*-*tert*-butylamodiaquine (**F**)[6] (Figure 1). Peculiar modification of the side chain of CQ is observed in ferroquine (**G**),[7] which incorporates a ferrocenyl moiety, and in piperaquine (**H**), an old bisquinoline analogue that has been resumed as an efficient partner of artemisinin derivatives.[8]

A valuable approach to obtain novel CQ analogues is represented by the assembly of hybrid molecules containing two pharmacophores joined through a linker that should not disturb the interaction of each component with the relevant target.[9] The two pharmacophores may act in different ways on the same target, as in trioxaquine derivatives **I** and **J**,[10] which are able to alkylate heme and to inhibit the formation of hemozoin, or they may act on different targets, as is the case for "reversed chloroquines" **K** and **L**; these derivatives incorporate different kinds of inhibitors of *P. falciparum* chloroquine resistance transporter (*Pf*CRTs), which inhibit the formation of hemozoin and also the efflux of the drug from the digestive vacuole of the parasite.[11]

Particularly interesting hybrid antimalarial agents have been obtained by combining the scaffold of chloroquine and primaquine (PQ) through different linkers (e.g., **M**).[12] Primaquine, an

[a] *Dr. B. Tasso, Dr. F. Novelli, Dr. M. Tonelli, Prof. F. Sparatore*
Dipartimento di Farmacia, Università degli Studi di Genova
Viale Benedetto XV 3, 16131 Genova (Italy)
E-mail: bruno.tasso@unige.it

[b] *Dr. A. Barteselli, Prof. A. Sparatore*
Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano
via Mangiagalli 25, 20133 Milano (Italy)

[c] *Dr. N. Basilico*
Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche
Università degli Studi di Milano
via C. Pascal 36, 20133 Milano (Italy)

[d] *Dr. S. Parapini, Prof. D. Taramelli*
Dipartimento di Scienze Farmacologiche e Biomolecolari
Università degli Studi di Milano
via C. Pascal, 36, 20133 Milano (Italy)

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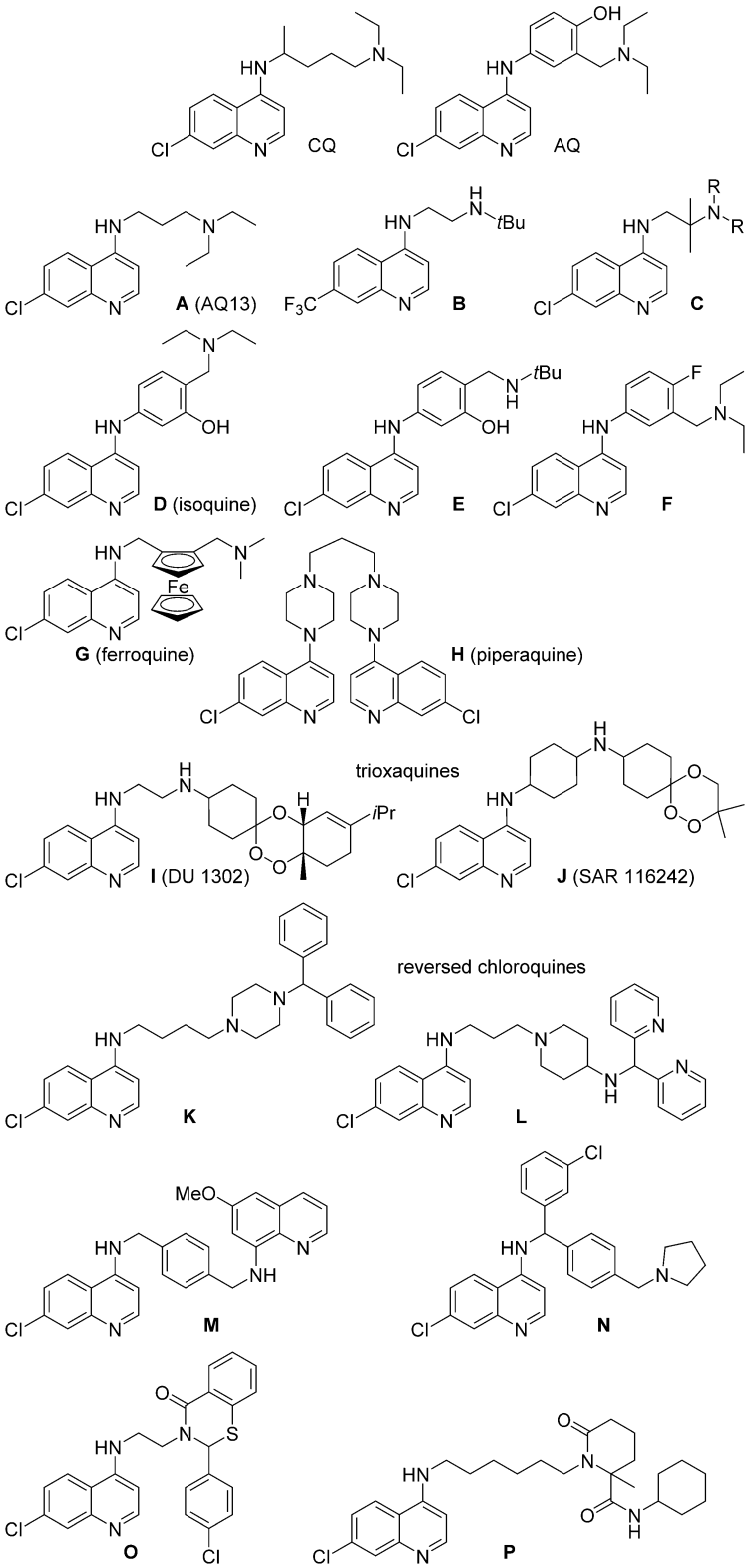


Figure 1. Examples of chloroquine (CQ) and amodiaquine (AQ) analogues with modified side chains.

old 8-aminoquinoline derivative, acts against all exoerythrocytic forms of *Plasmodium* and displays gametocytocidal activity, which is still the only transmission-blocking antimalarial that is clinically available.[13] Moreover, PQ synergizes chloroquine activity against CQ-R *P. falciparum* by inhibiting the *Pf*CRT.[14] The synthesized CQ–PQ hybrids display good to excellent activity against both the liver and blood stages of the *Plasmodium* infection and against gametocytes, which thus inhibits the transmission cycle.

Additional examples of hybrid molecules are combination products of the 4-aminoquinoline scaffold with polyarylmethyl system **N**; these hybrids are able to form radical intermediates that are toxic to the parasite, and additionally, they block the formation of hemozoin.[15] Finally, CQ analogues **O** and **P** bearing a side chain ending with bulky, nonbasic moieties are noteworthy.[16,17] Some of these compounds display potent activity against different strains of *P. falciparum*, which suggests that a basic side chain is not very essential for antiplasmodial activity, and this opens new possibilities for the design of novel chloroquine analogues. Interestingly, the former group of compounds[16] inhibits (similar to CQ) the formation of hemozoin, whereas the latter[17] inhibits, albeit weakly, falcipain 2 (a cysteine protease critical for hemoglobin catabolism) as an alternative or additional target for antimalarial activity.[18]

Over the past several years we have been engaged in the search of novel antimicrobial agents, and recently, we studied the antimalarial activity of some 4-aminoquinoline and 9-aminoacridine derivatives (e.g., **Q**–**T**; Figure 2) characterized by the presence of a bulky, strongly basic, and lipophilic bicyclic moiety, that is, a quinolizidine (octahydro-2*H*-quinolizine) or pyrrolizidine (hexahydro-1*H*-pyrrolizine) ring.[19–22] These moieties appear as interesting structural features that are able to overcome the resistance mechanism. Indeed, these novel CQ analogues exhibited high antimalarial activity against CQ-susceptible (CQ-S) and CQ-R strains of *Pf* in vitro through a CQ-like mechanism, and some of them also showed efficacy against *P. berghei* and *P. yoelii* if given orally or intraperitoneally in a murine standard four-day test.[23,24]

Pursuing our efforts to obtain novel antimalarial agents containing a bulky basic head, we deemed it interesting to investigate the effects on the activity of the following structural changes, operated either singularly or in combination: 1) Further elongation of the chain between the NH group and the quinolizidine nucleus (e.g., compounds **1**–**5**; Figure 3), which has already been shown to improve activity and/or to alter the selectivity for CQ-R or CQ-S parasites.[5a,19] 2) The shift in the side chain from the 1-position to the 9a-position of the quinolizidine ring to avoid stereochemical issues (e.g., compounds **6**–**8**; Figure 3). 3) The exchange of the quinolizidine nucleus with an even bulkier, but less lipophilic, moiety such as that of cytisine [(1*R*,5*S*)-(1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one] (e.g., compounds **9**–**20**; Figure 3). The pyridone ring present in the cytisine moiety might act as a Michael

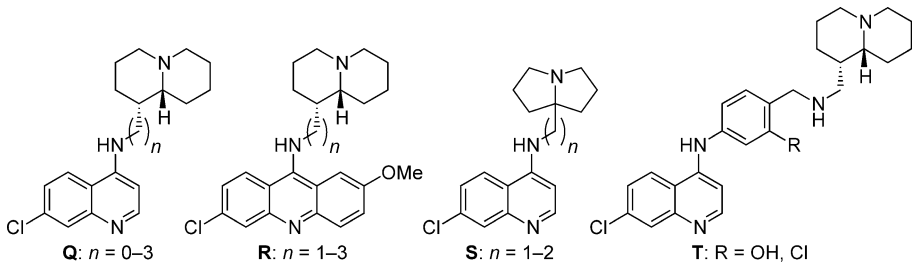


Figure 2. Previously studied 4-aminoquinoline and 9-aminoacridine derivatives with bulky basic head groups.

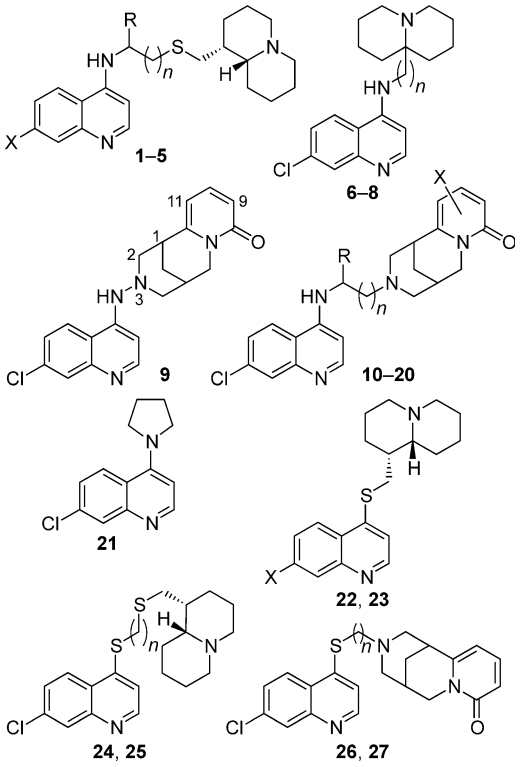


Figure 3. Structures of novel quinoline derivatives tested as antimalarial agents (see Table 1 for structural details).

acceptor and, as such, could affect the thiol group of the active site of cysteine protease (as falcipain).[25] Interestingly, some *N*-benzylcytisine derivatives have been recently shown to display activity against the parasite *Leishmania donovani*.[26] 4) The replacement, in the foregoing compounds, of the NH group with a sulfur bridge (e.g., compounds **22**–**27**; Figure 3), which in addition to decreasing the basicity of the molecule should also increase its lipophilicity. The right balance of these physicochemical characteristics is fundamental for the accumulation of the drug in the digestive vacuole and for its association with hematin to inhibit the formation of hemozoin.[4c] 7-Chloro-4-(pyrrolidin-1-yl)quinoline (**21**), incidentally obtained during the syntheses, was also considered. The structures of the 27 tested compounds are collected in Figure 3. On the basis of previous observations,[27–29] the bulky basic heads of the relevant compounds are thought to be fairly resistant to metabolic attack.

Results and Discussion

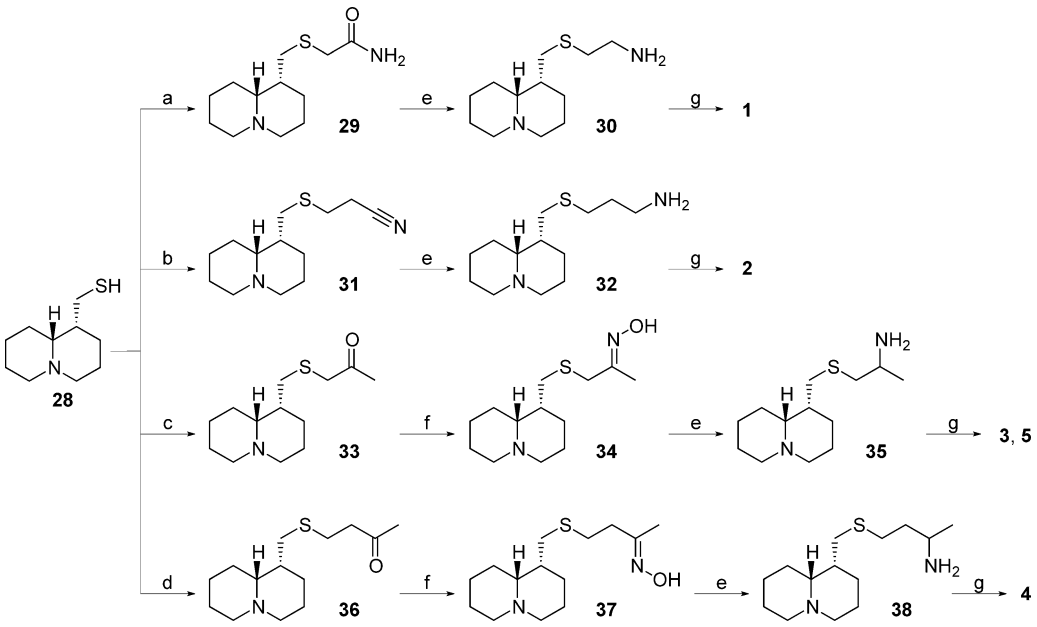
Chemistry

The preparation of the long-chain ω-(quinolizidin-1-yl)alkylamines required for the synthesis of higher homologues of compounds of general structure **Q** (Figure 2) may be a difficult and time-consuming task; thus, we resorted to the use of ω-(lupinylthio)alkylamines {ω-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)-methylthio]alkylamines} taking into account the well-known bioisosterism of a methylene unit for a sulfur atom. This approach was already used profitably for the preparation of long-chain *N*-(ω-quinolizindinyl)alkanoyl- and *N*- or *O*-(ω-quinolizidinyl)alkyl derivatives of several aromatic and heteroaromatic moieties (e.g., phenothiazine and other tricyclic systems, anilines, 9-aminoacridines, and 6-hydroxycoumarin), to obtain muscarinic M1 and M2 ligands,[30,31] antiarrhythmics,[32] antiprion toxicity agents,[33] and acetylcholinesterase and butyrylcholinesterase inhibitors.[34]

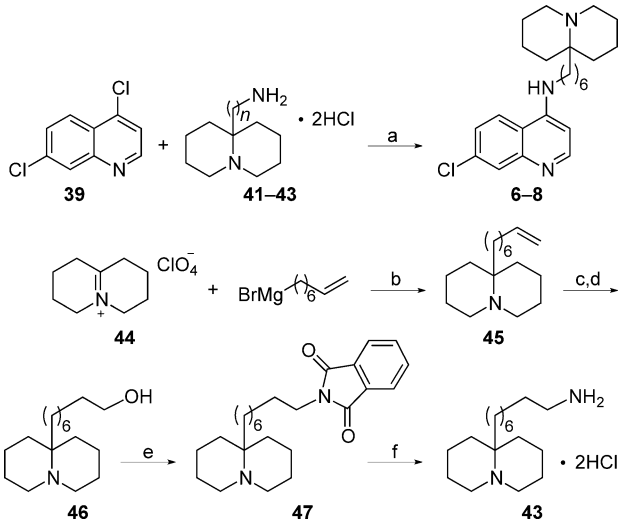
Thus, thiolupinine[35] (**28**) [(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methanthiol] was treated with iodoacetamide,[34a] acrylonitrile,[33] chloroacetone, and methyl vinyl ketone to obtain compounds **29**, **31**, **33**, and **36**, respectively. The last two ketones were converted into corresponding oximes **34** and **37**, which in addition to compounds **29** and **31** were reduced with $LiAlH_4$ to required long-chain amines **30**, **32**, **35**, and **38**. These amines were, finally, treated with 4,7-dichloroquinoline (**39**) or 4-chloro-7-trifluoromethylquinoline (**40**) to obtain compounds **1**–**5** (Scheme 1).

For the synthesis of compounds **6**–**8**, 4,7-dichloroquinoline was treated with ω-(quinolizidin-9a-yl)alkylamines (dihydrochlorides) **41**–**43** in the presence of diisopropylethylamine (DIPEA). 4/6-(Octahydro-2*H*-quinolizin-9a-yl)butan/hexan-1-amines (**41** and **42**) were recently described by Barteselli et al.,[36] and the homologue 8-(octahydro-2*H*-quinolizin-9a-yl)octan-1-amine (**43**) was obtained in a similar way starting from quinolizidinium perchlorate[37] through hydroxyalkylation with the method of McIntosh[38] and final amination according to Becker et al.[39] (Scheme 2).

Compound **9** was obtained by treating *N*-aminocytisine[40] (**48**) with 4,7-dichloroquinoline, whereas compounds **10**–**16**, **19**, and **20** were obtained from the reactions of 4-[*N*-(ω-bromoalkyl)amino]-7-chloroquinolines **54**–**58** with cytisine (**59**), 9-nitrocytisine (**60**),[41] and 9,11-dibromocytisine (**61**)[42] eventually in the presence of DIPEA. The reaction of 4-[*N*-(4-bromobutyl)amino]-7-chloroquinoline with cytisine or its derivatives produced, instead of the expected compounds, 7-chloro-4-(pyrrolidin-1-yl)quinoline (**21**) by intramolecular loss of hydrogen bromide. The catalytic reduction of 9-nitrocytisine derivatives **15** and **16** gave corresponding amino derivatives **17** and **18** (Scheme 3).



Scheme 1. *Reagents and conditions*: a) iodoacetamide, EtOH, RT, 3 days; b) acrylonitrile, Triton B, dioxane, 60 °C, 1 h; c) chloroacetone, EtOH, RT, 1 h; d) methyl vinyl ketone, dry dioxane, RT, 1 h; e) $LiAlH_4$, THF, reflux, 72 h; f) hydroxylamine hydrochloride/6 N NaOH, EtOH, reflux, 2 h; g) 4-chloro-7-substituted quinoline, phenol, 180 °C, 4 h.



Scheme 2. *Reagents and conditions*: a) phenol, DIPEA, N_2, 130 °C 5 h; b) $ZnBr_2$, dry THF, 50 °C→reflux, 20 h; c) 1 N BH_3 in THF, 2-methyl-2-butene, dry THF, RT, 4.5 h; d) H_2O, 6 N NaOH, 35% H_2O_2, RT, 1 h; e) phthalimide, Ph_3P, DEAD, dry THF, RT, 20 h; f) 6 N HCl, reflux, 20 h.

Required 4-[*N*-(ω-bromoalkyl)amino]-7-chloroquinolines **54**–**58** were obtained by treating 4,7-dichloroquinoline with the suitable ω-hydroxyalkylamines and subsequent bromination by means of 48% hydrobromic acid in the presence of concentrated sulfuric acid, as previously described for some of them.[43–46]

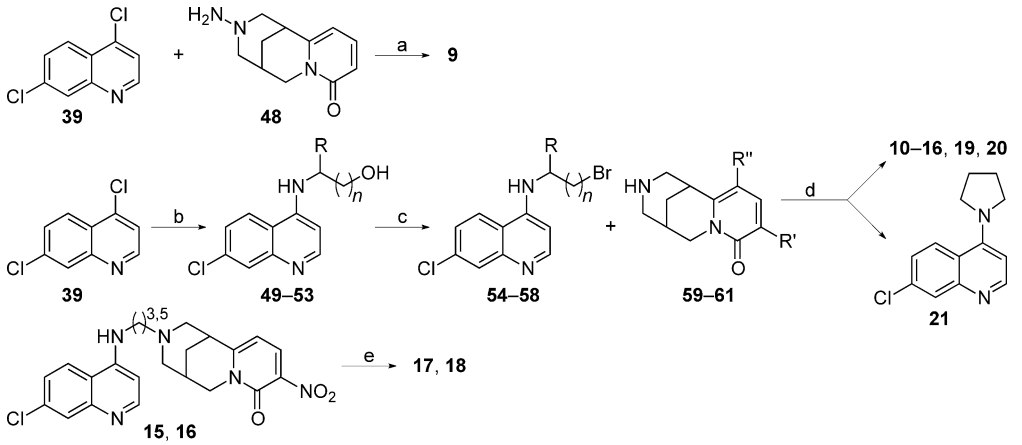
Compounds **22** and **23** were obtained by treating thiolupinine (**28**)[35] with the proper 4-chloro-7-substituted quinolines, whereas compounds **24**–**27** were obtained from 7-chloro-4-thioquinoline (**62**),[47] which was treated with *S*-(haloalkyl)thiolupinines **63** and **64**[32,48] and *N*-(ω-haloalkyl)cytisines **65** and **66**[49] (Scheme 4).

Antimalarial activity

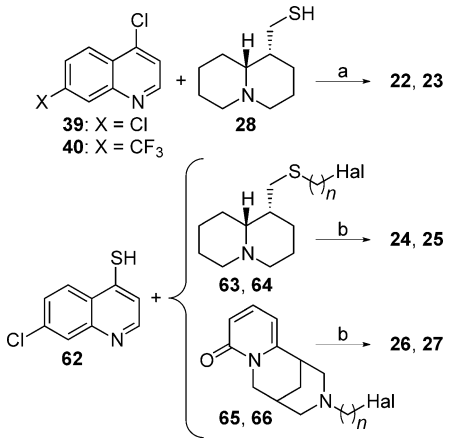
Compounds **1**–**27** were tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. The antimalarial activity was evaluated by inhibition of parasite growth, as measured by the production of parasite lactate dehydrogenase. Table 1 shows the IC_{50} values against the D-10 and W-2 strains of *P. falciparum*, as well as the means of the ratios between the IC_{50} of CQ and that of each compound against the D-10 and W-2 strains, calculated for each single experiment.

The ratios between the IC_{50} values of each compound against the two strains are also indicated. These last values are suggestive of the susceptibility of the drug to the resistance mechanism (resistance factor, RF). The results listed in Table 1 indicate that many of novel 4-aminoquinoline derivatives **1**–**20** display high antimalarial activity with IC_{50} values <100 nM against both the CQ-S and CQ-R strains, which thus resulted in 11 compounds that were from 2.7- to 13.4-fold more active than chloroquine against the CQ-R strain W-2. The remaining compounds show an appreciable level of activity with sub-micromolar IC_{50} values with the only exception of compounds **10** and **13**. On the other hand, 4-pyrrolidinylquinoline **21** and compounds **22**–**27**, which are six derivatives of 4-thioquinoline, display only modest or moderate antimalarial activity with IC_{50} values in the low (1.4–12.5) μM range; nevertheless, these compounds exhibit an interesting improvement in the resistance factor.

With regard to the structure–activity relationships, a general enhancement in the activity with increasing distance between



Scheme 3. *Reagents and conditions*: a) phenol, N_2, 180 °C 7 h; b) hydroxyalkylamine, 150 °C, 3 h; c) 48% HBr/H_2SO_4, 160 °C, 3.5 h; d) CH_3CN, 100 °C, 36 h; e) EtOH, H_2, 10% Pd/C, RT.



Scheme 4. *Reagents and conditions*: a) Dowtherm A, 170 °C, 2 h; b) KOH, CH_3CN, N_2, RT, 24 h.

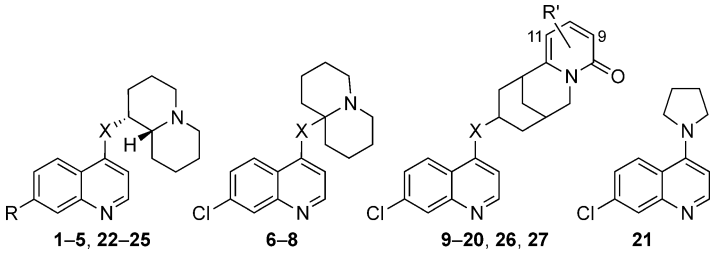
the quinoline ring and the basic nitrogen atom of the side chain was observed, even if with some peculiarities in each subgroup of compounds. Indeed, we previously showed[19] that the increasing length of the methylene chain in compounds of structure **Q** (Figure 2) influenced negatively the antimalarial activity, especially against the CQ-R strain (for $n=0$, $IC_{50}=23.3$ nM; for $n=3$, $IC_{50}=384.4$ nM). Such a trend is now inverted in compounds **1** and **2**, which are characterized by a longer linker of four and five atoms, respectively. This behavior is similar to that observed in a set of diethylaminoalkyl analogues of CQ that first exhibit decreasing activity in the medium-length compounds but then recover the high activity if the number of methylene units is further increased.[5a]

Interestingly, although stereochemical issues are overcome by shifting the linker (between the quinoline and quinolizidine moieties) from the 1-position to the 9a-position of the quinolizidine ring, this shift produces only minor variations in the antimalarial potency (compare compounds **1** and **2** with **6** and **7**). However, if the linker is elongated up to eight methylene units (as in **8**), a strong increase in potency is observed ($IC_{50}=$ 9 and 25.9 nM for the CQ-S and CQ-R strains, respectively), even if the interest in this compound is restrained by its complex synthesis.

In the set of cytisinyl derivatives **9**–**13**, elongation of the linker produces an alternating effect on the activity, which is initially decreasing and then steadily increasing. The minimal activity is observed in compound **10** (linker with two methylene units), with IC_{50} values of 941 and 1776 nM for the CQ-S and CQ-R strains, respectively. The maximal activity is found in compound **12** (linker with five methylene units) with IC_{50} values of 32.9 and 77.1 nM for the CQ-S and CQ-R strains, which are 28- and 23-fold enhancements in potency, respectively. The introduction of substituents on the pyridone ring of the cytisine moiety (i.e., compounds **14**–**20**) produces a clear increase in antimalarial activity that is cumulative with the positive effect of elongation of the linker. Thus, compounds **16**, **18**, and **20** are the most potent among those presently reported, and these derivatives are 5-, 10-, and 8-fold more potent than chloroquine, respectively, and are from 1/3 to 2/3 as potent as artemisinin, against the W-2 (CQ-R) strain of *P. falciparum*. Moreover, the cytisinyl derivatives are characterized by low cytotoxicity (Table 2) versus two human cell lines (human microvascular endothelial cells, HMEC-1, and human Caucasian hepatocellular carcinoma, HepG2), with selectivity indices (SI) in the ranges of 400 to 880 and of 143 to 540, respectively (CQ, $SI>120$ and 62, respectively). Additionally, comparably with CQ and artemisinin, they produced less than 10% hemolysis upon incubation for 72 h at a concentration of 200 μM with uninfected erythrocytes. Among the most potent compounds, only **8** displayed some hemolytic activity with a CC_{50} value of (148.6 ± 1.2) μM.

Therefore, the cytisinyl derivatives of 4-aminoquinoline appear worthy of further evaluation as antimalarial agents, also in consideration of the easy availability of the starting material, which is extracted from the seeds of the widespread *Laburnum anagyroides* and of other plants (*Sophora* and *Thermopsis* species). Particularly, the complex structure of the cytisine moiety that characterizes the best compounds warrants thorough investigation of their mechanism of action. The high activity of

Table 1. Antimalarial activity of compounds **1**–**27** tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) *P. falciparum* strains.



1–5, **22–25** **6–8** **9–20**, **26**, **27** **21**

Compd	R	X	R′	D-10 (CQ-S)		W-2 (CQ-R)		Ratio IC_{50}
				IC_{50} [nM][a]	Ratio to CQ[b]	IC_{50} [nM][a]	Ratio to CQ[b]	CQ-R/CQ-S[c]
1	Cl	NH-CH_2-CH_2-S-CH_2	–	53.7 ± 13.5	0.61	259.4 ± 60.1	3.15	4.83
2	Cl	NH-$(CH_2)_3$-S-CH_2	–	27.1 ± 6.9	1.21	155.4 ± 49.0	5.25	5.72
3	Cl	NH-CH(CH_3)-CH_2-S-CH_2	–	24.6 ± 8.3	1.25	82.6 ± 15.5	6.20	3.36
4	Cl	NH-CH(CH_3)-CH_2-CH_2-S-CH_2	–	23.1 ± 8.7	1.33	119.9 ± 48.3	4.27	5.19
5	CF_3	NH-CH(CH_3)-CH_2-S-CH_2	–	56.1 ± 17.3	0.55	83.5 ± 37.8	6.13	1.49
6	–	NH-$(CH_2)_4$	–	22.5 ± 9.6	0.80	475.3 ± 145.7	0.68	21.12
7	–	NH-$(CH_2)_6$	–	27.15 ± 8.0	1.50	194.3 ± 74.6	1.67	7.16
8	–	NH-$(CH_2)_8$	–	9.0 ± 3.7	2.34	25.9 ± 3.7	13.45	2.88
9	–	NH	H	370.1 ± 110	0.06	635.8 ± 113	0.50	1.72
10	–	NH-CH_2-CH_2	H	941.5 ± 262.1	0.02	1775.9 ± 304.4	0.16	1.89
11	–	NH-$(CH_2)_3$	H	116.5 ± 38.4	0.17	298.7 ± 35.7	0.94	2.56
12	–	NH-$(CH_2)_5$	H	32.9 ± 2.4	0.28	77.1 ± 22.8	2.96	2.34
13	–	NH-CH(CH_3)-CH_2	H	902.3 ± 263.4	0.01	1048.6 ± 360.8	0.22	1.16
14	–	NH-CH_2-CH_2	9-NO_2	115.3 ± 23.2	0.08	874.8 ± 366.2	0.26	7.59
15	–	NH-$(CH_2)_3$	9-NO_2	62.7 ± 5.3	0.15	546.6 ± 174.3	0.42	8.72
16	–	NH-$(CH_2)_5$	9-NO_2	16.5 ± 5.2	0.56	42.0 ± 21.1	5.43	2.55
17	–	NH-$(CH_2)_3$	9-NH_2	96.2 ± 7.5	0.14	478.4 ± 140.0	0.52	4.975
18	–	NH-$(CH_2)_5$	9-NH_2	11.9 ± 6.0	1.11	24.3 ± 10.1	10.32	2.04
19	–	NH-$(CH_2)_3$	9,11-Br_2	47.5 ± 13.9	0.28	90.7 ± 42.1	2.76	1.91
20	–	NH-$(CH_2)_5$	9,11-Br_2	16.8 ± 5.5	0.79	31.1 ± 16.4	8.06	1.85
21	–	–	–	886.6 ± 25.9	0.02	810.9 ± 162.8	0.31	0.915
22	Cl	S-CH_2	–	7190.0 ± 2620	0.003	4530.0 ± 2557	0.07	0.63
23	CF_3	S-CH_2	–	> 10000	NA[d]	7820.0 ± 2539	0.04	0.62
24	Cl	S-CH_2-CH_2-S-CH_2	–	2412.0 ± 192.1	0.013	1818.0 ± 660.1	0.28	0.75
25	Cl	S-$(CH_2)_3$-S-CH_2	–	1747.0 ± 63.0	0.018	1416.0 ± 379.8	0.36	0.81
26	–	S-$(CH_2)_3$	H	9841.0 ± 2023	0.003	12281.0 ± 400.3	0.04	1.25
27	–	S-$(CH_2)_4$	H	3922.0 ± 162.2	0.008	5190.0 ± 2028	0.10	1.32
chloroquine (CQ)				20.0 ± 5	–	320 ± 51	–	16
mefloquine				33.2 ± 11.3	–	16.9 ± 7.3	–	0.51
artemisinin				27.7 ± 8.9	–	14.5 ± 4.2	–	0.52

[a] Results are expressed as the mean ± SD of at least three different experiments, each performed in duplicate or triplicate. [b] Mean of ratios between the IC_{50} of CQ and that of each compound against *P. falciparum* strains D-10 or W-2, calculated for each single experiment. [c] Resistance factor: Ratios between the IC_{50} values of each compound against the two strains of *P. falciparum*. [d] Not available.

these compounds might be related to strong association of the molecules with hematin, similar to that observed for Solomon's[16] compounds (e.g., **O**, Figure 1) bearing a side chain with a bulky, highly lipophilic head. However, owing to the Michael acceptor characteristics of the pyridone ring embodied in the cytisine moiety, interaction with the thiol group of the active site of falcipain cannot be excluded. Actually, even compound **P** (Figure 2) inhibited, albeit weakly, falcipain 2, which should attack one of its carboamide groups.[17]

Notably, pyridone-derived falcipain inhibitors have been described,[50] for which, however, the pyridone ring served mainly as a rigid peptidomimetic backbone, and additional more potent electrophilic groups were included in the structures. In both sets of quinolizidinylalkyl and cytisinylalkyl derivatives, branching of the linker produces a slight increase in activity, as shown by comparison of the activities of unbranched compounds **1**, **2**, and **10** with those of corresponding methyl homologues **3**, **4**, and **13**.

Replacement of the chlorine atom in the quinoline ring with a trifluoromethyl group decreases the activity on the CQ-S strain, but it does not affect the activity on the CQ-R strain (compare compounds **3** and **5**). Such a decrease in activity was already observed by us[19] in compounds of structure **Q** (Figure 2) and by several authors in other 4-aminoquinoline derivatives. Kaschula et al.[4c] correlated the decreased activity with the stronger electron-withdrawing character of the trifluoromethyl group, which decreases the basicity of both nitrogen atoms of the side chain. However, in the case of the CQ-R strain the enhanced lipophilicity, and subsequent cell penetration, of the fluorinated compounds might compensate the negative effect of the decreased basicity on the activity.[51]

Table 2. Cytotoxicity against human microvascular endothelial cells (HMEC-1) and human Caucasian hepatocyte carcinoma (HepG2), and selectivity index of the most potent and other representative compounds.

Compd	IC_{50} [nM][a]			SI[b]	
	HMEC-1	HepG2	W-2 (CQ-R)	HMEC-1	HepG2
3	18673 ± 5915	9773 ± 1912	82.6 ± 15.5	226	118.3
6	> 26000	NT	475.3 ± 145.7	> 54.7	–
7	9211 ± 938	NT	194.3 ± 74.6	47.4	–
8	5495 ± 697	1869 ± 628	25.9 ± 3.7	212.2	72.2
12	54800 ± 14547	11165 ± 2263	77.1 ± 22.8	710.8	144.8
16	23010 ± 6797	10113 ± 217	42.0 ± 21.1	547.8	240.8
18	21388 ± 5245	13142 ± 1402	24.3 ± 10.1	880.5	540.8
20	12413 ± 2066	4454 ± 1211	31.1 ± 16.4	399.1	143.8
24	75282 ± 11316	NT	1818.0 ± 660.1	41.4	–
CQ	> 38000	19942 ± 1643	320 ± 51	> 118.8	62.3
Arte	NT	> 106260	14.5 ± 4.2	–	> 7328.3

[a] Results are the mean ± SD of three experiments each performed in duplicate; NT: not tested. [b] Selectivity index: [IC_{50}(HMEC-1 or HepG2)]/[IC_{50}(W-2(CQ-R)].

Conclusions

Twenty-seven novel chloroquine analogues characterized by a side chain bearing a bulky basic head (i.e., octahydro-2*H*-quinolizine and 1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one) were synthesized and tested for antimalarial activity against the D-10 (CQ-susceptible) and W-2 (CQ-resistant) strains of *P. falciparum*. Most compounds were active against both strains with nanomolar or sub-micromolar IC_{50} values; in particular, 11 compounds were 2.7- to 13.4-fold more active than chloroquine against the chloroquine-resistant strain.

The influence on activity of some structural features was analyzed, and in each subset of compounds, the positive effect of increasing the length of the spacer between the quinoline and the bulky basic head was confirmed. Moreover, exchange of the 4-NH group with a sulfur bridge maintained the antimalarial activity but at a lower level of potency; however, this structural modification produced an interesting improvement in the resistance factor (RF = 0.6–0.8) that appears worthy of further investigation. The introduction of a cytisinyl head and a spacer containing five methylene units produced the most interesting compounds endowed with high activity, low cytotoxicity on two human cell lines (HMEC-1 and HepG2), and nonhemolytic at concentrations up to 200 μM. Therefore, these compounds deserve further investigation to define their ADME profiles and their in vivo antimalarial activities in a murine standard four-day test.

Finally, in compounds **22**–**27** the replacement of the 4-NH group by a sulfur bridge should increase the lipophilicity of the molecule but still allow resonance of the positive charge from the protonated quinoline nitrogen atom to the sulfur bridge, as suggested by the UV spectra of other 7-chloro-4-thioquinoline derivatives described by Clinton and Suter.[52] The presence of this resonance should favor interaction of the molecules with ferriprotopophirin IX (Fe^{III}PPIX)[4a] and should therefore increase antimalarial activity, even if no activity was observed upon screening the above 4-S analogues of CQ in vivo against avian malaria.[52] Actually, the lack of in vivo activity may be related to inadequate dosage or unfavorable pharmacokinetic characteristics of the particular compounds.

Indeed, S-bridged compounds **22**–**27** display, in vitro, moderate antimalarial activity against both of the CQ-S and CQ-R strains of *P. falciparum*, with most IC_{50} values in the low-micromolar range. This activity is in line with that exhibited by a set of 7-chloro-4-(*S*-diethylaminoalkyl)thioquinolines (described by Natarajan et al.[53]), despite the largely different bulkiness of the terminal heads of the respective side chains.

In both series of compounds, the activity improves somewhat with elongation of the linker, in conformity with the trend observed for the respective NH analogues. However, differently from the latter, most of these sulfur-containing compounds exhibit a resistance factor (RF = IC_{50} CQ-R/IC_{50} CQ-S) significantly below 1 (RF = 0.62–0.81 for compounds **22**–**25**), which suggests low susceptibility of the S-bridged compounds to resistance mechanisms. Thus, once more, within a given type of compounds the enhanced lipophilicity seems to balance the negative effect of the reduced basicity on the activity against the CQ-R strain. However, comparing the S-bridged compounds with the 4-aminoquinoline derivatives, the reduced activities of the former appear to be mainly due to the strongly decreased basicity of the quinoline nitrogen atom (mean $pK_{a2} = 6.40 \rightarrow pK_{a2} = 3.18$), not balanced by the increased lipophilicity (Table 3, values calculated with ACD/Labs 7.00).

Table 3. Calculated log*D* and pK_a values for representative compounds (quinolizidine derivatives: **Q**-**8** and **22**–**25**; cytisine derivatives: **11**–**20** and **26**, **27**).

Compd	$\log D_{7.4}$[b]	$\log D_{5.2}$[c]	pK_{a1}[d]	pK_{a2}[e]	pK_{a3}[f]
NH bridged					
Q ($n = 1$)	1.98	0.73	10.18	6.35	−4.48
1	2.67	1.41	10.15	6.30	−4.69
2	3.01	1.73	10.16	6.43	−4.13
3	3.02	1.79	10.15	6.23	−3.99
5	3.70	2.84	NC[h]	NC[h]	NC[h]
8	4.13	2.92	10.59	6.51	−3.66
11	2.40	−0.35	8.80	6.32	−4.94
12	2.88	0.23	8.92	6.48	−3.86
16	2.82	−0.12	8.88	6.48	−3.86
18[g]	2.32	−0.43	8.78	6.48	−3.84
20	4.47	1.44	8.08	6.48	−3.83
S bridged					
22	2.81	2.13	9.91	3.30	–
23	2.99	2.32	9.91	2.49	–
24	3.31	2.79	10.13	3.25	–
25	3.65	3.15	10.15	3.37	–
26	3.21	1.29	8.07	3.28	–
27	3.33	1.51	8.38	3.37	–

[a] Calculated with ACD/Labs ver. 7.00. [b] Log*D* at plasma pH. [c] Log*D* at *P. falciparum* digestive vacuole pH. [d] pK_a of side chain N atom. [e] pK_a of quinoline N atom. [f] pK_a of 4-NH group. [g] pK_a of 9-NH_2 group: 3.61. [h] Not calculated.

Experimental Section

Chemistry

General: All commercially available solvents and reagents were used without further purification unless otherwise stated. Melting points were determined with a Büchi apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Gemini 200 or Varian Mercury 300VX spectrometer in $CDCl_3$ or [D_6]DMSO with Me_4Si as an internal standard; in the assignments, Q denotes the quinolizidine ring and bisp denotes the bispidine moiety. HRMS was performed with a FT-Orbitrap mass spectrometer in positive electrospray ionization (ESI). Elemental analyses were performed with a Carlo Erba EA-1110 CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmacy of Genoa University. All final compounds and some intermediates were characterized by ^{1}H NMR spectroscopy and elemental analyses or HRMS, which indicated that their purity was always $\geq$95%. The remaining intermediates were characterized by either ^{1}H NMR spectra or elemental analyses. For the most interesting compounds (i.e., **12**, **16**, **18**, and **20**) the ^{13}C NMR spectroscopy data are also included.

General method for the preparation of 4-(*N*-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]alkyl}amino)-7-substituted quinolines 1–5: Amino compound **30**, **32**, **35**, or **38** (6.5 mmol) was mixed with equimolar amounts of 4,7-dichloroquinoline (1.28 g) or 4-chloro-7-trifluoromethylquinoline (1.50 g) and phenol (4 g). The mixture was heated at 180 °C for 4 h. After cooling, the mixture was treated with 2 N NaOH solution until strongly alkaline and was extracted with Et_2O (3×40 mL). The Et_2O solution was washed with 2 N NaOH solution, then with H_2O, and finally with 5% acetic acid solution (2×20 mL). The acid solution was alkalized with 2 N NH_3 solution (to pH 8) and was extracted with CH_2Cl_2 (3× 50 mL) to yield a light-brown solid that was washed with boiling dry Et_2O (5 mL), which left the title compound as white crystals. By concentration of the Et_2O solution, some more compound was recovered. In the cases of compounds **4** and **5**, the extracted raw compounds were chromatographed on neutral alumina (1:20) eluting with CH_2Cl_2.

7-Chloro-4-(*N*-{2-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]ethyl}amino)quinoline (1): Yield: 37%; mp: 160–165 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.03–2.14 (m, 14H, Q), 2.64–2.91 (m, 2H, 2H_a near N of Q+2H, SCH_2), 2.93 (t, J = 6.2 Hz, 2H, CH_2S), 3.42–3.61 (m, 2H, Ar-NH-CH_2), 5.66 (s, 1H, NH, collapsed with D_2O), 6.44 (d, J = 5.4 Hz, 1H, arom), 7.41 (dd, J = 9.0, 2.0 Hz, 1H, arom), 7.50 (d, J = 9.0 Hz, 1H, arom), 7.98 (d, J = 2.0 Hz, 1H, arom), 8.56 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{21}H_{28}ClN_3S$ (389.99): C 64.68, H 7.24, N 10.77, S 8.22, found: C 64.76, H 7.36, N 10.81, S 8.27.

7-Chloro-4-(N-{3-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]prop-1-yl}amino)quinoline (2): Yield: 38%; mp: 95–100 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.04–2.14 (m, 14H, Q+2H, $CH_2CH_2CH_2$), 2.69 (t, J = 6.4 Hz, 2H, CH_2S), 2.76–2.93 (m, 2H, 2H_a near N of Q+2H, SCH_2), 3.49–3.58 (m, 2H, Ar-NH-CH_2), 5.60 (s, 1H, NH, collapsed with D_2O), 6.44 (d, J = 5.4 Hz, 1H, arom), 7.36 (dd, J = 9.0, 2.0 Hz, 1H, arom), 7.61 (d, J = 9.0 Hz, 1H, arom), 7.96 (d, J = 2.0 Hz, 1H, arom), 8.53 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{22}H_{30}ClN_3S$ (404.01): C 65.40, H 7.48, N 10.40, S 7.49, found: C 65.22, H 7.75, N 10.34, S 7.89%.

7-Chloro-4-(*N*-{3-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]prop-2-yl}amino)quinoline (3): Yield: 50%; mp: 110–112 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.88–2.12 (m, 14H, Q+d superimposed at 1.44, J = 6.2 Hz, 3H, CH-CH_3), 2.48–2.96 (m, 2H_a near N of Q+2H, SCH_2+2H, CH_2S), 3.72–4.08 (m, 1H, CH-CH_3), 5.33 (s, 1H, NH, collapsed with D_2O), 6.45 (d, J = 5.4 Hz, 1H, arom), 7.39 (dd, J = 8.8, 2.0 Hz, 1H, arom), 7.72 (d, J = 8.8 Hz, 1H, arom), 7.97 (d, J = 2.0 Hz, 1H, arom), 8.55 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{22}H_{30}ClN_3S$ (404.01): C 65.40, H 7.48, N 10.40, S 7.94, found: C 65.26, H 7.54, N 10.37, S 8.06.

7-Chloro-4-(*N*-{4-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]but-2-yl}amino)quinoline (4): Yield: 9%; mp: 77–82 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.95–2.14 (14H, Q+2H, CH$CH_2$$CH_2$+ d superimposed at 1.36, J = 6.4 Hz, 3H, CH-CH_3), 2.42–2.96 (m, 2H_a near N of Q+2H, SCH_2+2H, CH_2S), 3.74–4.08 (m, 1H, CH-CH_3), 5.20 (s, 1H, NH, collapsed with D_2O), 6.50 (d, J = 5.6 Hz, 1H, arom), 7.37 (dd, J = 9.0, 1.6 Hz, 1H, arom), 7.72 (d, J = 9.0 Hz, 1H, arom), 7.97 (d, J = 2.0 Hz, 1H, arom), 8.53 ppm (d, J = 5.6 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{32}ClN_3S$ (418.04): C 66.08, H 7.72, N 10.05, S 7.67, found: C 66.15, H 7.89, N 10.08, S 7.67.

4-(*N*-{3-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]prop-2-yl}amino)-7-trifluoromethylquinoline (5): Yield: 59%; mp: 91–93 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.92–2.15 (14H, Q+d superimposed at 1.46, J = 6.6 Hz, 3H, CH-CH_3), 2.47–2.96 (m, 2H_a near N of Q+2H, SCH_2+2H, CH_2S), 3.78–4.12 (m, 1H, CH-CH_3), 5.41 (s, 1H, NH, collapsed with D_2O), 6.55 (d, J = 5.4 Hz, 1H, arom), 7.62 (dd, J = 8.8, 1.6 Hz, 1H, arom), 7.91 (d, J = 8.8 Hz, 1H, arom), 8.29 (s, 1H, arom), 8.65 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{30}F_3N_3S$ (425.55): C 63.13, H 6.91, N 9.60, S 7.33, found: C 63.04, H 7.03, N 9.70, S 7.15.

General method for the preparation of 7-chloro-4-{[ω-octahydro-2*H*-quinolizin-9a-yl)alkyl]amino}quinolines 6–8: A mixture of amine **41**, **42**,[36] or **43** as the dihydrochloride or free base (0.88 mmol), 4,7-dichloroquinoline (192 mg, 0.97 mmol), phenol (415 mg, 4.41 mmol), and DIPEA (0.25 mL, 1.76 mmol) was heated under a N_2 atmosphere for 5 h at 130 °C. After cooling, the mixture was diluted with CH_2Cl_2, and the resulting solution washed with 2 N NaOH solution (3×5 mL) and then with brine (10 mL). After drying (Na_2SO_4), the solvent was evaporated, and the crude solid was purified by column chromatography (silica gel, different mixtures CH_2Cl_2/MeOH as indicated for each compound). For the preparation of compound **8**, it was more convenient to use the free base (obtained preliminary from the dihydrochloride), which avoided the use of DIPEA and heating the mixture for only 3 h.

7-Chloro-4-{[4-(octahydro-2*H*-quinolizin-9a-yl)butyl]amino}quinoline (6): Column chromatography (CH_2Cl_2/MeOH 94:6); solid washed with PE. Yield: 64%; mp: 157.8–159.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.19–1.84 (m, 14H, Q+4H, CH_2CH_2), 2.42–2.64 (m, 2H, 2H_a near N of Q+2H, CH_2C), 3.31–3.37 (m, 2H, Ar-NH-CH_2), 4.96 (s, 1H, NH), 6.43 (d, J = 5.5 Hz, 1H, arom), 7.36 (dd, J = 2.2, 8.8 Hz, 1H, arom), 7.65 (d, J = 8.8 Hz, 1H, arom), 7.95 (d, J = 2.2 Hz, 1H, arom), 8.54 ppm (d, J = 5.5 Hz, 1H, arom); elemental analysis calcd (%) for $C_{22}H_{30}ClN_3$ (371.95): C 71.04, H 8.13, N 11.30, found: C 70.89, H 8.28, N 11.45.

7-Chloro-4-{[6-(octahydro-2*H*-quinolizin-9a-yl)hexyl]amino}quinoline (7): Column chromatography (CH_2Cl_2/MeOH 92:8); amorphous solid crystallized and washed with Et_2O. Yield: 46%; mp: 144.6–146.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.12–1.82 (m, 14H, Q+8H, $CH_2CH_2CH_2CH_2$), 2.41–2.65 (m, 2H, 2H_a near N of Q+2H, CH_2C), 3.29–3.35 (m, 2H, Ar-NH-CH_2), 4.96 (s, 1H, NH), 6.42 (d, J = 5.5 Hz, 1H, arom), 7.36 (dd, J = 2.2, 8.8 Hz, 1H, arom), 7.65 (d, J = 8.8 Hz, 1H, arom), 7.96 (d, J = 2.2 Hz, 1H, arom), 8.53 ppm (d, J = 5.2 Hz, 1H, arom); elemental analysis calcd (%) for $C_{24}H_{34}ClN_3$ (400.00): C 72.06, H 8.57, N 10.51, found: C 72.23, H 8.81, N 10.19.

7-Chloro-4-{[8-(octahydro-2*H*-quinolizin-9a-yl)octyl]amino}quinoline (8): Column chromatography (CH_2Cl_2/MeOH 94:6); amorphous solid crystallized and washed with Et_2O. Yield: 55%; mp: 116.8–118.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.12–1.77 (m, 14H, Q + 12H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.47–2.63 (m, 2H, 2H$_a$ near N of Q + 2H, CH_2C), 3.28–3.32 (m, 2H, Ar-NH-CH_2), 4.95 (s, 1H, NH), 6.42 (d, J = 5.5 Hz, 1H, arom), 7.36 (dd, J = 2.2, 8.8 Hz, 1H, arom), 7.65 (d, J = 8.8 Hz, 1H, arom), 7.96 (d, J = 2.2 Hz, 1H, arom), 8.54 ppm (d, J = 5.2 Hz, 1H, arom); elemental analysis calcd (%) for $C_{26}H_{38}ClN_3$ (428.05): C 72.95, H 8.95, N 9.82, found: C 73.14, H 9.08, N 9.56. The compound was converted into the corresponding dihydrochloride; mp: 250–252 °C (dec.); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.05–1.91 (m, 26H), 2.86–3.09 (m, 2H), 3.35–3.40 (m, 2H), 3.49–3.52 (m, 2H), 6.85 (d, J = 7.2 Hz, 1H, arom), 7.76 (d, J = 8.8 Hz, 1H, arom), 8.06 (s, 1H, arom), 8.52 (d, J = 7.2 Hz, 1H, arom), 8.70 (d, J = 8.8 Hz, 1H, arom), 9.62 (br s, 1H, collapsed with D_2O), 10.20 ppm (br s, 2H, collapsed with D_2O); elemental analysis calcd (%) for $C_{26}H_{38}ClN_3$·2HCl (500.98): C 62.33, H 8.05, N 8.39, found: C 62.01, H 8.32, N 8.15.

7-Chloro-4-(cytisin-3-yl)aminoquinoline (9): A mixture of *N*-aminocytisine[40] (332 mg, 1.62 mmol), 4,7-dichloroquinoline (333 mg, 1.62 mmol), and phenol (1.03 g, 4.41 mmol) was heated under a N_2 atmosphere for 7 h at 180 °C. After cooling, the mixture was taken up with 2 N NaOH to strongly alkaline pH and then with Et_2O (3× 20 mL). The insoluble compound was filtered and washed with 2 N NaOH (3 mL), H_2O (2×3 mL), and thoroughly with Et_2O (3×5 mL) and finally crystallized from EtOH. Yield: 94%; mp: 296–298 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO) δ = 1.70–1.98 (m, 2H, bisp), 2.54 (s, 1H, bisp), 2.92–3.05 (m, 2H, bisp), 3.08–3.25 (m, 2H, bisp), 3.30 (s, 1H, bisp), 3.75 (dd, J = 5.5, 14.6 Hz, 1H, bisp) 4.04 (d, J = 14.6 Hz, 1H, bisp), 6.02 (d, J = 4.4 Hz, 1H, α-pyr), 6.13 (d, J = 6.3 Hz, 1H, α-pyr), 6.33 (d, J = 8.8 Hz, 1H, arom), 7.30–7.53 (m, 1H, α-pyr + 1H, arom), 7.74 (s, 1H, arom), 8.09 (d, J = 8.8 Hz, 1H, arom), 8.14 (d, J = 5 Hz, 1H, arom), 8.47 ppm (s, 1H, NH collapsed with D_2O); HRMS (ESI): *m/z*: calcd for $C_{20}H_{20}ClN_4O$: 367.13256 $[M+H]^+$, found: 367.13245.

General method for the preparation of 7-chloro-4-{*N*-[ω-(citisin-3-yl)alkyl]amino}quinolines 10–13: In an Aldrich pressure tube, cytisine (0.19 g, 1 mmol) was dissolved in CH_3CN (3 mL) and 4-[(ω-bromoalkyl)amino]-7-chloroquinoline **54–58** (0.5 mmol) was added;[43–46] the closed tube was heated at 100 °C for 36 h. The cooled mixture was filtered from cytisine hydrobromide, and the organic phase was evaporated to dryness. The residue was taken up in H_2O (10 mL), alkalized (2 N NaOH until strongly alkaline), and extracted with CH_2Cl_2 (3×40 mL). After drying (Na_2SO_4), the solvent was removed, and the oily residue was subjected to chromatography on alumina (1:25) eluting with CH_2Cl_2.

7-Chloro-4-{*N*-[2-(citisin-3-yl)ethyl]amino}quinoline (10): Yield: 78%; mp: 79–81 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.77–2.10 (m, 3H, bisp), 2.34–2.92 (m, 2H, NH-CH_2 + 3H, bisp), 2.94–3.33 (m, 2H, Ar-NH-CH_2 + 2H, bisp), 4.01 (dd, J = 15.6, 6.4 Hz, 1H, bisp), 4.23 (d, J = 15.6 Hz, 1H, bisp), 5.36 (s, 1H, NH, collapses with D_2O), 5.95 (d, J = 6.8 Hz, 1H, α-pyr), 6.28 (d, J = 5.4 Hz, 1H, arom), 6.50 (d, J = 9.2 Hz, 1H, α-pyr), 7.04 (d, J = 9.2 Hz, 1H, α-pyr), 7.12–7.23 (m, 1H, arom), 7.46 (dd, J = 8.8, 1.6 Hz, 1H, arom), 7.91 (d, J = 1.6 Hz, 1H, arom), 8.49 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{22}H_{23}ClN_4O$·0.75H_2O (408.41): C 64.69, H 6.05, N 13.79, found: C 64.85, H 6.25, N 13.56.

7-Chloro-4-{*N*-[3-(citisin-3-yl)propyl]amino}quinoline (11): Yield: 74%; mp: 143–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.65–2.10 (m, 4H, bisp + 2H $CH_2CH_2CH_2$), 2.24–2.61 (m, 2H, NH-CH_2 + 2H, bisp), 2.86–3.23 (m, 2H, Ar-NH-CH_2 + 2H, bisp), 3.86–3.93 (dd, J = 15.6, 6.4 Hz, 1H, bisp), 4.13–4,18 (d, J = 15.6 Hz, 1H, bisp), 4.97 (s, 1H, NH, collapses with D_2O), 6.01 (d, J = 6.9 Hz, 1H, α-pyr), 6.20 (d, J = 5.5 Hz, 1H, arom), 6.46 (d, J = 9.0 Hz, 1H, α-pyr), 7.27–7.42 (m, 1H, α-pyr + 1H, arom), 7.56 (dd, J = 8.8, 1.9 Hz, 1H, arom), 7.91 (d, J = 1.9 Hz, 1H, arom), 8.45 ppm (d, J = 5.5 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{25}ClN_4O$ (408.92): C 67.55, H 6.16, N 13.70, found: C 67.65, H 6.45, N 13.38.

7-Chloro-4-{*N*-[5-(citisin-3-yl)pentyl]amino}quinoline (12): Yield: 80%; mp: 61–64 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.86–1.58 (m, 2H, bisp + 6H, $CH_2CH_2CH_2$), 1.62–2.06 (m, 2H, bisp), 2.12–2.53 (m, 2H, NH-CH_2 + 3H, bisp), 2.71–3.32 (m, 2H, Ar-NH-CH_2 + 1H, bisp), 3.84 (dd, J = 15.2, 6.4 Hz, 1H, bisp), 4.19 (d, J = 15.2 Hz, 1H, bisp), 5.85 (s, 1H, NH, collapsed with D_2O), 6.04 (d, J = 6.8 Hz, 1H, α-pyr), 6.34 (d, J = 5.14 Hz, 1H, arom), 6.45 (dd, J = 9.2, 1.4 Hz, 1H, α-pyr), 7.21–7.37 (m, 1H, α-pyr + 1H, arom), 7.39 (dd, J = 8.8, 1.8 Hz, 1H, arom), 7.96 (d, J = 1.8 Hz, 1H, arom), 8.12 (d, J = 8.6 Hz, 1H, arom), 8.49 ppm (d, J = 5.4 Hz, 1H, arom); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 162.80, 150.94, 150.26, 149.46, 147.58, 137.95, 133.89, 126.81, 124.12, 121.37, 116.30, 115.20, 103.92, 97.56, 59.71, 58.98, 55.70, 49.22, 42.55, 34.62, 27.06, 26.94, 24.93, 24.61, 23.92 ppm; elemental analysis calcd (%) for $C_{25}H_{29}ClN_4O$ (436.98): C 68.71, H 6.69, N 12.82, found: C 68.89, H 6.90, N 12.81.

7-Chloro-4-{*N*-[2-(citisin-3-yl)-1-methylethyl]amino}quinoline (13): Yield: 53%; mp: 221–223 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.13 (d, J = 6.4 Hz, 3H, CHCH_3), 1.63–2.02 (m, 3H, bisp), 2.33–2.66 (m, 2H, NH-CH_2 + 2H, bisp), 2.77–3.12 (m, 3H, bisp), 3.55–3.78 (m, 1H, CHCH$_3$), 3.82–4.04 (m, 2H, bisp), 5.27 (s, 1H, NH, collapsed with D_2O), 6.06 (d, J = 6.8 Hz, 1H, α-pyr), 6.34 (d, J = 5.6 Hz, 1H, arom), 6.53 (dd, J = 9.2, 1.4 Hz, 1H, α-pyr), 7.18–7.44 (m, 2H, arom, + 1H, α-pyr), 7.94 (d, J = 0.8 Hz, 1H, arom), 8.49 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{25}ClN_4O$ (408.92): C 67.55, H 6.16, N 13.70, found: C 67.72, H 6.56, N 13.42.

General method for the preparation of 7-chloro-4-{[ω-(9-nitrocytisin-3-yl)alkyl]amino}quinolines 14–16 and 7-chloro-4-{[ω-(9,11-dibromocytisin-3-yl)alkyl}quinolines 19 and 20: In an Aldrich pressure tube, the substituted cytisine[41,42] (0.61 mmol) was dissolved in CH_3CN (4 mL). Subsequently, equimolar amounts of DIPEA and 4-[(ω-bromoalkyl)amino]-7-chloroquinoline **54–58** were sequentially added;[43–46] the closed tube was heated at 100 °C for 24 h. After cooling, the solvent was evaporated, and the residue was taken up with H_2O (10 mL), alkalized, (2 N NaOH until strongly alkaline), and extracted with CH_2Cl_2 (3×40 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness; the residue was finally chromatographed on alumina (1:30) eluting with CH_2Cl_2. The obtained compound was, eventually, further purified by trituration with dry Et_2O.

7-Chloro-4-{[2-(9-nitrocytisin-3-yl)ethyl]amino}quinoline (14): Yield: 66%; mp: 182–184 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.86–2.12 (m, 2H, bisp), 2.43–2.90 (m, 2H, NH-CH_2 + 2H, bisp), 2.98–3.37 (m, 2H, Ar-NH-CH_2 + 3H, bisp), 4.07 (dd, J = 16.2, 6.6 Hz, 1H, bisp), 4.32 (d, J = 16.2 Hz, 1H, bisp), 5.16 (s, 1H, NH, collapsed with D_2O), 6.04 (d, J = 8.2 Hz, 1H, α-pyr), 6.28 (d, J = 5.4 Hz, 1H, arom), 7.07 (d, J = 9.0 Hz, 1H, arom), 7.38 (dd, J = 8.0, 2.0 Hz, 1H, arom), 7.92 (d, J = 2.0 Hz, 1H, arom), 8.17 (d, J = 8.0 Hz, 1H, α-pyr), 8.49 ppm (d, J = 5.4 Hz, 1H arom); elemental analysis calcd (%) for $C_{22}H_{22}ClN_5O_3$ (439.90): C 60.07, H 5.04, N 15.92, found: C 60.17, H 5.33, N 15.96.

7-Chloro-4-{[3-(9-nitrocytisin-3-yl)propyl]amino}quinoline (15): Yield: 77%; mp: 103–105 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.65–2.08 (m, 2H, bisp + 2H, $CH_2CH_2CH_2$), 2.32–2.62 (m, 2H, NH-CH_2 + 2H, bisp), 2.91–3.22 (m, 2H, Ar-NH-CH_2 + 3H, bisp), 4.01 (dd, J = 16.0, 6.4 Hz, 1H, bisp), 4.24 (d, J = 16.0 Hz, 1H, bisp), 4.98 (s, 1H,

NH, collapsed with D_2O), 6.13 (d, J=8.2 Hz, 1H, α-pyr), 6.24 (d, J=5.4 Hz, 1H, arom), 7.36 (dd, J=9.0, 2.2 Hz, 1H, α-pyr), 7.64 (d, J=9.0 Hz, 1H, arom), 7.96 (d, J=2.2 Hz, 1H, arom), 8.33 (d, J=8.2 Hz, 1H, α-pyr), 8.51 ppm (d, J=5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{24}ClN_5O_3 \cdot 0.5\,H_2O$ (462.93): C 59.67, H 5.44, N 15.13, found: C 59.94, H 5.75, N 14.75.

7-Chloro-4-{[5-(9-nitrocytisin-3-yl)pentyl]amino}quinoline (16): Yield: 74%; mp: 84–86 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.82–2.04 (m, 2H, bisp+6H, $CH_2CH_2CH_2$), 2.08–2.62 (m, 2H, NH-CH_2+2H, bisp), 2.82–3.35 (m, 2H, Ar-NH-CH_2+3H, bisp), 3.94 (dd, J=16.0, 6.4 Hz, 1H, bisp), 4.22 (d, J=16.0 Hz, 1H, bisp), 5.44 (s, 1H, NH, collapsed with D_2O), 6.11 (d, J=8.2 Hz, 1H, α-pyr), 6.34 (d, J=5.4 Hz, 1H, arom), 7.36 (dd, J=9.0, 2.2 Hz, 1H, α-pyr), 7.82–7.99 (m, 2H, arom), 8.32 (d, J=8.2 Hz, 1H, α-pyr), 8.51 ppm (d, J=5.4 Hz, 1H, arom); ^{13}C NMR (50 MHz, $CDCl_3$): δ=159.87, 154.30, 149.90, 149.41, 147.07, 136.73, 134.12, 133.54, 126.54, 124.29, 121.02, 116.08, 101.76, 97.66, 58.77, 58.54, 55.66, 50.39, 42.24, 35.52, 28.63, 27.22, 26.57, 24.89, 24.22, 23.62 ppm; elemental analysis calcd (%) for $C_{25}H_{28}ClN_5O_3$ (481.98): C 62.30, H 5.86, N 14.53, found: C 62.33, H 5.87, N 14.25.

7-Chloro-4-{[3-(9,11-dibromocytisin-3-yl)propyl]amino}quinoline (19): Yield: 68%; mp: 103–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.47–1.98 (m, 4H, bisp+2H, $CH_2CH_2CH_2$), 2.24–2.53 (m, 2H, NH-CH_2+2H, bisp), 2.94–3.13 (m, 2H, Ar-NH-CH_2+1H, bisp), 3.96 (dd, J=16.0, 6.2 Hz, 1H, bisp), 4.17 (d, J=16.0 Hz, 1H, bisp), 4.86 (s, 1H, NH, collapsed with D_2O), 6.21 (d, J=5.5 Hz, 1H, arom), 7.37 (dd, J=8.8, 2.0 Hz, 1H, arom), 7.58 (d, J=8.8 Hz, 1H, arom), 7.82 (s, 1H, α-pyr), 7.95 (d, J=2.0 Hz, 1H, arom), 8.54 ppm (d, J=5.5 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{23}Br_2ClN_4O$ (566.72): C 48.75, H 4.09, N 9.89, found: C 48.67, H 4.19, N 9.63.

7-Chloro-4-{[5-(9,11-dibromocytisin-3-yl)pentyl]amino}quinoline (20): Yield: 69%; mp: 104–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.92–2.01 (m, 4H, bisp+6H, $CH_2CH_2CH_2$), 2.08–2.52 (m, 2H, NH-CH_2+1H, bisp), 2.74–3.52 (m, 2H, Ar-NH-CH_2+3H, bisp), 3.92 (dd, J=16.0, 6.2 Hz, 1H, bisp), 4.20 (d, J=16.0 Hz, 1H, bisp), 5.41 (s, 1H, NH, collapsed with D_2O), 7.21 (d, J=5.5 Hz, 1H, arom), 7.39 (dd, J=9.0, 2.2 Hz, 1H, arom), 7.86 (s, 1H, α-pyr), 7.95 (d, J=2.0 Hz, 1H, arom), 8.50 ppm (d, J=5.5 Hz, 1H, arom); ^{13}C NMR (50 MHz, $CDCl_3$): δ=157.92, 149.68, 147.66, 146.92, 142.69, 134.27, 126.49, 124.39, 121.07, 116.09, 111.29, 97.70, 96.03, 58.49, 55.91, 55.64, 51.72, 42.47, 33.89, 27.25, 26.82, 24.86, 23.79 ppm; elemental analysis calcd (%) for $C_{25}H_{27}Br_2ClN_4O \cdot 0.5\,H_2O$ (603.78): C 49.72, H 4.67, N 9.18, found: C 49.60, H 5.04, N 8.92.

7-Chloro-4-(pyrrolidin-1-yl)quinoline (21): Reaction of 4-[(4-bromobutyl)amino]-7-chloroquinoline with cytisine or 9-nitrocytisine, either in the presence or absence of DIPEA, only the intramolecular loss of hydrogen bromide was obtained to give. Yield: 88%; mp: 90–91 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ=1.82–2.21 (m, 4H, CH_2CH_2), 3.42–3.56 (m, 4H, N-CH_2), 6.44 (d, J=5.4 Hz, 1H, arom), 7.28 (dd, J=9.2, 2.2 Hz, 1H, arom), 7.95 (d, J=2.2 Hz, 1H, arom), 8.16 (d, J=9.2 Hz, 1H, arom), 8.47 ppm (d, J=5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{13}H_{13}ClN_2$ (232.71): C 67.10, H 5.63, N 12.04%, found: C 67.31, H 5.88, N 12.10%.

General method for the preparation of 4-{*N*-[ω-(9-aminocytisin-3-yl)alkyl]amino}-7-chloroquinolines 17 and 18: Nitro compound **15** or **16** (0.25–0.44 mmol) was dissolved in EtOH (40 mL), and 10% palladium on charcoal (20 mg) was added. The mixture was hydrogenated at atmospheric pressure. The catalyst was filtered, and the solvent was removed under vacuum. The residue was rinsed with dry Et_2O (3 mL) to give the title compound as a white powder that rapidly became pale yellow.

4-{*N*-[3-(9-Aminocytisin-3-yl)propyl]amino}-7-chloroquinoline (17): Yield: 86%; mp: 173–175 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=0.78–1.98 (m, 4H, bisp+2H, $CH_2CH_2CH_2$), 2.04–2.45 (m, 2H, NH-CH_2+1H, bisp), 2.69–3.58 (m, 2H, Ar-NH-CH_2+3H, bisp), 3.74 (dd, J=16.0, 6.2 Hz, 1H, bisp), 3.95 (d, J=16.0 Hz, 1H, bisp), 4.67 (s, 1H, NH, collapsed with D_2O), 5.89 (d, J=7.4 Hz, 1H, α-pyr), 6.12 (d, J=5.5 Hz, 1H, arom), 6.43 (d, J=7.4 Hz, 1H, α-pyr), 7.15 (s, 2H, NH_2, collapsed with D_2O) 7.40 (dd, J=9.0, 1.8 Hz, 1H, arom), 7.72 (d, J=1.8 Hz, 1H, arom), 8.21 (d, J=9.0 Hz, 1H, arom), 8.34 ppm (d, J=5.5 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{26}ClN_5O$ (423.94): C 65.16, H 6.18, N 16.52, found: C 64.72, H 6.38, N 16.29.

4-{*N*-[5-(9-Aminocytisin-3-yl)pentyl]amino}-7-chloroquinoline (18): Yield: 76%; mp: 137–139 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=0.78–2.01 (m, 4H, bisp+6H, $CH_2CH_2CH_2$), 2.11–2.42 (m, 2H, NH-CH_2+1H, bisp), 2.64–3.49 (m, 2H, Ar-NH-CH_2+3H, bisp), 3.73 (dd, J=16.0, 6.2 Hz, 1H, bisp), 3.90 (d, J=16.0 Hz, 1H, bisp), 4.76 (s, 1H, NH, collapsed with D_2O), 5.84 (d, J=7.4 Hz, 1H, α-pyr), 6.32–6.47 (m, 1H, arom+1H, α-pyr), 7.19 (s, 2H, NH_2, collapsed with D_2O) 7.40 (dd, J=9.0, 2.2 Hz, 1H, arom), 7.74 (d, J=2.0 Hz, 1H, arom), 8.24 (d, J=9.0 Hz, 1H, arom), 8.36 ppm (d, J=5.5 Hz, 1H, arom); ^{13}C NMR (50 MHz, $CDCl_3$): δ=157.23, 150.27, 149.43, 147.58, 138.19, 133.89, 133.20, 126.86, 124.10, 121.17, 116.31, 112.67, 103.59, 97.59, 60.13, 59.03, 55.31, 49.52, 42.44, 33.99, 28.63, 27.16, 26.87, 25.55, 24.50, 23.61 ppm; elemental analysis calcd (%) for $C_{25}H_{30}ClN_5O$ (451.99): C 66.43, H 6.69, N 15.49, found: C 66.72, H 6.62, N 15.12.

General method for the preparation of 4-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl)thio]-7-substituted quinolines 22 and 23: A suspension of 4,7-dichloroquinoline (0.51 g, 2.6 mmol) or 4-chloro-7-trifluoromethylquinoline (0.6 g) in Dowtherm A (4 mL) was heated at 100 °C until a clear solution was obtained, and then a solution of thiolupinine[35] (0.48 g, 2.6 mmol) in Dowtherm A (1 mL) was added. The tube was closed and the temperature was raised and maintained at 170 °C for 2 h. After cooling, the pasty material was partitioned between Et_2O and 0.5 N HCl (30 mL each). The aqueous phase was extracted with Et_2O (2×10 mL), alkalized, (2 N NaOH (20 mL) and extracted again with Et_2O (3×40 mL). The dried Et_2O solution was evaporated to leave an oil that slowly crystallized.

7-Chloro-4-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl)thio]-quinoline (22): Yield: 61%; mp: 114–115 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ=1.16–2.28 (m, 14H, Q), 2.75–3.02 (m, 2H, CH_2S), 3.18–3.54 (m, 2H_a near N of Q), 7.21 (d, J=5.0 Hz, 1H, arom), 7.47 (dd, J=9.0, 2.2 Hz, 1H, arom), 8.03–8.08 (m, 2H, arom), 8.67 ppm (d, J=5.0 Hz, 1H, arom); elemental analysis calcd (%) for $C_{19}H_{23}ClN_2S$ (346.92): C 65.78, H 6.68, N 8.07, found: C 66.02, H 6.78, N 8.11. Elemental analysis calcd (%) for $C_{19}H_{23}ClN_2S \cdot HCl \cdot 0.5\,H_2O$ (392.39): C 58.16, H 6.42, N 7.14, found: C 58.45, H 6.36, N 7.13.

4-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}-7-trifluoromethylquinoline (23): Yield: 69%; mp: 78–79 °C (pentane); ^{1}H NMR (200 MHz, $CDCl_3$) δ=1.06–2.24 (m, 14H, Q), 2.70–2.99 (m, 2H, CH_2S), 3.14–3.50 (m, 2H_a near N of Q), 7.32 (d, J=5.5 Hz, 1H, arom), 7.69 (dd, J=8.8, 2.0 Hz, 1H, arom), 8.25 (d, J=8.8 Hz, 1H, arom), 8.35 (s, 1H, arom), 8.77 ppm (d, J=5.5 Hz, 1H, arom); elemental analysis calcd (%) for $C_{20}H_{23}F_3N_2S$ (380.47): C 63.14, H 6.09, N 7.36, found: C 63.18, H 6.10, N 7.35.

General method for the preparation of *S*-(7-Chloro-4-quinolinyl)-ω-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}alkanethiols 24 and 25: A mixture of 7-chloro-4-thioquinoline[47] (0.21 g, 1.09 mmol) and KOH (ground pellets, 61 mg, 1.09 mmol) in CH_3CN (5 mL) was stirred for 30 min under an atmosphere of nitrogen. A solution of an equimolar amount of *S*-(2-chloroethyl)thiolupinine[32]

(**63**) or *S*-(3-bromopropyl)thiolupinine[48] (**64**) in CH_3CN (3 mL) was dropwise added slowly, and the solution was stirred at room temperature for 24 h. The solvent was removed, and the residue was taken up in 1 N HCl (10 mL). The acid solution was extracted with Et_2O (2×10 mL), alkalized (2 N NaOH, 15 mL), and extracted with CH_2Cl_2 (3×10 mL). The dried solution was evaporated, and the oily residue was subjected to chromatography on alumina (1:30) eluting with CH_2Cl_2.

***S*-(7-Chloroquinolin-4-yl)-2-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}ethanethiol (24)**: Yield: 43%. Yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.04–2.12 (m, 14H, Q), 2.66–2.98 (m, 2H, CH_2S + 2H, ArSCH_2S + 2H, SCH_2), 3.14–3.50 (m, 2H, 2H$_a$ near N of Q), 7.19 (dd, *J* = 4.7, 1.6 Hz, 1H, arom), 7.49 (dd, *J* = 9.0, 2.0 Hz, 1H, arom), 7.96–8.15 (m, 2H, arom), 8.72 ppm (dd, *J* = 4.7, 1.6 Hz, 1H, arom); elemental analysis calcd (%) for $C_{21}H_{27}ClN_2S_2$ (407.04): C 61.97, H 6.69, N 6.88, S 15.76, found: C 61.80, H 6.75, N 6.83, S 15.28.

***S*-(7-Chloroquinolin-4-yl)-3-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}propanethiol (25)**: Yield: 40%; mp: 81–83 °C (dry Et_2O); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95–2.21 (m, 14H, Q + 2H, $CH_2CH_2CH_2$), 2.56–2.95 (m, 2H, CH_2S + 2H, ArSCH_2S + 2H, SCH_2), 3.11–3.35 (m, 2H, 2H$_a$ near N of Q), 7.21 (d, *J* = 5.0 Hz, 1H, arom), 7.49 (dd, *J* = 8.8, 2.0 Hz, 1H, arom), 7.97–8.12 (m, 2H, arom), 8.70 ppm (d, *J* = 5.0 Hz, 1H, arom); elemental analysis calcd (%) for $C_{22}H_{29}ClN_2S_2$ (421.06): C 62.75, H 6.94, N 6.65, S 15.23, found: C 62.51, H 7.19, N 6.68, S 15.38. Elemental analysis calcd (%) for $C_{22}H_{29}ClN_2S_2$·HCl·1.25 H_2O (405.90): C 55.07, H 6.77, N 5.84, S 13.36, found: C 55.21, H 6.93, N 5.56, S 11.98.

General method for the preparation of 7-chloro-4-{*S*-[ω-(citisin-3-yl)alkyl]thio}quinolines 26 and 27: A mixture of 7-chloro-4-thioquinoline[47] (0.28 g, 1.43 mmol) and KOH (ground pellets, 80 mg, 1.43 mmol) in CH_3CN (5 mL) was stirred for 30 min under an atmosphere of nitrogen. A solution of an equimolar amount of *N*-(2-chloroethyl)cytisine[49] (**65**) or *N*-(4-chloropropyl)cytisine[49] (**66**) in CH_3CN (5 mL) was dropwise slowly added, and the solution was stirred at room temperature for 72 h. The solvent was removed, and the residue was taken up in 1 N HCl (5 mL). The acid solution was filtered, alkalized (2 N NaOH until strongly alkaline) and extracted with CH_2Cl_2 (3×20 mL). The dried solution was concentrated, and the oily residue was subjected to chromatography on alumina (1:30) eluting with CH_2Cl_2 and CH_2Cl_2/MeOH (98:2). Finally, the solid was crystallized with dry Et_2O.

7-Chloro-4-{*S*-[3-(citisin-3-yl)propyl]thio}quinoline (26): Yield: 54%; mp: 140–141 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.78–2.16 (m, 3H, bisp + 2H, $CH_2CH_2CH_2$), 2.41–2.72 (2H, S-CH_2 + 3H, bisp), 2.84–3.21 (m, 2H, NH-CH_2 + 2H, bisp), 4.04 (dd, *J* = 15.6, 6.4 Hz, 1H, bisp), 4.29 (d, *J* = 15.6 Hz, 1H, bisp), 6.12 (d, *J* = 6.8 Hz, 1H, α-pyr), 6.51 (d, *J* = 5.4 Hz, 1H, arom), 7.11 (d, *J* = 5.4 Hz, 1H, arom), 7.32 (m, 1H, arom), 7.64 (dd, *J* = 8.8, 1.6 Hz, 1H, arom), 8.07–8.28 (m, 2H, arom), 8.84 ppm (d, *J* = 5.0 Hz, 1H, arom); elemental analysis calcd (%) for $C_{23}H_{24}ClN_3OS$ (425.97): C 64.85, H 5.68, N 9.86, S 7.53, found: C 64.66, H 5.66, N 10.05, S 7.53.

7-Chloro-4-{*S*-[4-(citisin-3-yl)butyl]thio}quinoline (27): Yield: 43%; mp: 100–101 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.56–2.17 (m, 2H, bisp + 4H, $CH_2CH_2CH_2CH_2$), 2.33–2.66 (2H, S-CH_2 + 3H, bisp), 2.88–3.19 (m, 2H, NH-CH_2 + 2H, bisp), 4.02 (dd, *J* = 15.6, 6.4 Hz, 1H, bisp), 4.22 (d, *J* = 15.6 Hz, 1H, bisp), 6.11 (d, *J* = 6.8 Hz, 1H, α-pyr), 6.56 (d, *J* = 9.0 Hz, 1H, arom), 7.22 (d, *J* = 5.0 Hz, 1H, arom), 7.26–7.42 (m, 1H, arom), 7.65 (dd, *J* = 9.0, 2.2 Hz, 1H, arom), 8.15–8.31 (m, 2H, arom), 8.84 ppm (d, *J* = 5.0 Hz, 1H, arom); elemental analysis calcd (%) for $C_{24}H_{26}ClN_3OS$ (440.00): C 65.51, H 5.96, N 9.55, S 7.29, found: C 65.42, H 5.86, N 9.54, S 7.43.

ω-(Octahydro-quinolizin-1/9a-yl)alkylamines, intermediates for compounds 1–8: Amines **29**, **32**, **41**, and **42** were prepared as previously described by Tasso et al.,[32] Villa et al.,[33] and Barteselli et al.,[36] whereas amine **35**, **38**, and **43** were prepared as follows.

1-{[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizin-1-yl)methyl]thio}-2-oxopropane (33): Chloroacetone (0.75 mL, 6.5 mmol) was added to a solution of thiolupinine[35] (1 g, 5.4 mmol) in EtOH (5 mL) under protection from air, and the mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was partitioned between Et_2O (10 mL) and 1 N HCl (20 mL). The acid solution was alkalized with 2 N NaOH (15 mL) and extracted with Et_2O (4×10 mL). The Et_2O solution was dried (Na_2SO_4) and concentrated to leave a pale-yellow oil (1.23 g, 95%); elemental analysis calcd (%) for $C_{13}H_{23}NOS$ (241.39): C 64.68, H 9.60, N, 5.80, found: C 64.81, H 9.61, N 5.65.

2-Hydroxyimino-1-{[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizin-1-yl)methyl]thio}propane (34): 6 N NaOH (1.1 mL) was added to a solution of ketone **33** (1.23 g, 5.1 mmol) and hydroxylamine hydrochloride (0.43 g, 6.12 mmol) in EtOH (4 mL), and the solution was heated at reflux for 2 h and then evaporated under reduced pressure. The residue was taken up in H_2O (10 mL) and extracted with CH_2Cl_2 (3×10 mL). After removing the solvent, a pale-yellow oil (1.28 g, 97%) that crystallized on standing was recovered; mp: 79–80 °C (pentane); elemental analysis calcd (%) for $C_{13}H_{24}N_2OS$ (256.41): C 60.89, H 9.43, N 10.93, found: C 61.15, H 9.44, N 10.86.

2-Amino-1-{[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizin-1-yl)methyl]thio}propane (35): A solution of oxime **34** (0.90 g, 3.5 mmol) in dry Et_2O (20 mL) was added dropwise to a stirred and ice-cooled suspension of lithium aluminum hydride (0.65 g, 17 mmol) in dry Et_2O (20 mL), and then the suspension was heated at reflux for 20 h. After cooling, H_2O (1 mL), 15% NaOH solution (1 mL), and H_2O (1.5 mL) were sequentially added. The precipitate was filtered and washed thoroughly with Et_2O (3×10 mL), and the organic solution was dried with KOH pellets. After elimination of the solvent, the oily residue was distilled in vacuo (0.07 hPa) collecting a colorless oil (0.73 g, 86%) at 140 °C (air bath temperature); elemental analysis calcd (%) for $C_{13}H_{26}N_2S$ (242.42): C 64.40, H 10.81, N 11.56, found: C 64.57, H 10.97, N 11.52.

1-{[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizin-1-yl)methyl]thio}-3-oxobutane (36): A solution of methyl vinyl ketone (1.4 mL, 17.5 mmol) in dry dioxane (1.5 mL) was added dropwise to a solution of thiolupinine[35] (3.04 g, 16.3 mmol) in dry dioxane (2.5 mL). The solution was stirred at room temperature for 1 h and then maintained at 70 °C for 30 min, always under an atmosphere of nitrogen. After cooling, the solution was diluted with Et_2O (20 mL) and extracted with 0.5 N HCl (3×15 mL). The acid solution was strongly alkalized (2 N NaOH (30 mL)) and extracted with Et_2O (3×20 mL). After removing the solvent, the oily residue was distilled at 0.07 hPa collecting a pale-yellow oil (3.92 g, 93%) at 140 °C (air bath temperature); elemental analysis calcd (%) for $C_{14}H_{25}NOS$ (255.42): C 65.86, H 9.87, N 5.48, found: C 65.76, H 9.99, N 5.63.

3-Hydroxyimino-1-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}butane (37): Ketone **35** (3.48 g, 13.6 mmol) was converted into the corresponding oxime by treatment with hydroxylamine hydrochloride (1.14 g, 16.3 mmol) and 6 N NaOH solution (2.7 mL) under the conditions used for oxime **34**. Thus, a pale-yellow oil (3.55 g, 96%) was obtained. Elemental analysis calcd (%) for

$C_{14}H_{26}N_2OS$ (270.43): C 62.17, H 9.69, N 10.36, found: C 62.22, H 9.78, N 10.36.

3-Amino-1-{[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]thio}-butane (38): Following the procedure described for the preparation of amino compound **35**, oxime **37** (2.25 g, 8.3 mmol) was reduced with lithium aluminum hydride (1.57 g, 41 mmol). Thus, after distillation at 0.07 hPa (140 °C, air bath temperature) amine **38** (1.92 g, 90%) was obtained. Elemental analysis calcd (%) for $C_{14}H_{28}N_2S$ (256.45): C 65.57, H 11.00, N 10.93, found: C 65.33, H 11.34, N 10.74.

9a-(Oct-7-enyl)-octahydro-2*H*-quinolizine (45): A solution of 8-bromo-1-octene (3 g, 15.7 mmol) in anhydrous THF (4.5 mL) was added dropwise to a mixture of granular magnesium (15.7 mmol) and a catalytic amount (30 mg) of zinc bromide in dry THF (9 mL). After the addition of a crystal of iodine to trigger the reaction, the mixture was stirred for 4 h at 50 °C under an atmosphere of nitrogen. Once the Grignard compound was completely formed, dry THF (30 mL) and octahydroquinolizidinium perchlorate[37] (1.89 g, 7.85 mmol) were added, and the mixture was heated at reflux for 20 h. After cooling, the mixture was diluted with saturated aqueous solution of NH_4Cl (9 mL) and acidified with 6 N HCl (to pH 2). The aqueous layer was extracted with Et_2O (3×10 mL), then alkalized and saturated with K_2CO_3 and extracted several times with Et_2O (4×20 mL). The collected organic layer was dried (Na_2SO_4) and concentrated to dryness to give a yellow oil (790 mg, 54%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20–2.26 (m, 22H), 2.39–2.92 (m, 6H), 4.91–5.03 (m, 2H), 5.77–5.82 ppm (m, 1H).

8-(Octahydro-2*H*-quinolizin-9a-yl)octan-1-ol (46): A solution of compound **45** (750 mg) in anhydrous THF (13.5 mL) was added dropwise over 30 min to an ice-cooled mixture of 1 M borane–THF complex solution (12.6 mL, 12.6 mmol) and 2-methyl-2-butene (1.76 g, 70.1 mmol) diluted with dry THF (2.4 mL). The mixture was stirred at room temperature for 4.5 h and then diluted with H_2O (3.7 mL), 6 N NaOH (12.5 mL), and H_2O_2 (35% solution, 3.1 mL). The mixture was stirred at room temperature for 1 h and then saturated with K_2CO_3 and extracted with Et_2O (4×20 mL). The collected organic layer was concentrated to dryness, and the residue was diluted with Et_2O (50 mL) and extracted with 4 N HCl (3×30 mL). The aqueous layer was alkalized, saturated with K_2CO_3, and extracted with Et_2O (4×20 mL). The organic phase was dried (Na_2SO_4) and concentrated to dryness to give an oil that was purified by column chromatography (neutral Al_2O_3, grade IV; PE/Et_2O in gradient). Oil (380 mg, 48%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.08–1.69 (m, 26H), 2.38–2.42 (m, 2H), 2.55–2.64 (m, 2H), 3.62–3.66 ppm (m, 2H). One H is not visible, probably because of its interaction with the solvent.

2-[8-(Octahydro-2*H*-quinolizin-9a-yl)octyl]isoindoline-1,3-dione (47): Diethylazodicarboxylate (DEAD; 0.42 mL diluted with 1.1 mL of anhydrous THF) was added dropwise to an ice-cooled mixture of the above alcohol (340 mg, 1.27 mmol), phthalimide (374 mg, 2.54 mmol), triphenylphosphine (700 mg, 2.67 mmol), and dry THF (3.9 mL). The resulting mixture was stirred at room temperature for 20 h. The solvent was evaporated, and the crude oil was purified by column chromatography (silica gel; CH_2Cl_2/MeOH/conc. NH_3 96:3.6:0.4). Pale-yellow oil (490 mg, 96%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.09–1.67 (m, 24H), 2.52–2.66 (m, 4H), 3.48–3.70 (m, 4H), 7.69–7.73 (m, 2H arom), 7.81–7.85 ppm (m, 2H, arom).

8-(Octahydro-2*H*-quinolizin-9a-yl)octane-1-amine dihydrochloride (43): The above phthalimide derivative **47** (478 mg, 1.20 mmol) was dissolved in 6 N HCl (4.7 mL) and heated at reflux for 20 h. After ice cooling, the precipitated phthalic acid was filtered and washed with ice-cold H_2O (2×5 mL). The aqueous solution was concentrated under vacuum to dryness to afford a brownish, very hygroscopic powder (398 mg, 84%); ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.01–1.93 (m, 24H), 2.72–3.45 (m, 8H), 7.99 (br s, 2H, collapsed with D_2O), 10.27 ppm (br s, 2H, collapsed with D_2O). Free base: pale yellow oil; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 0.94–1.03 (m, 4H), 1.25–1.51 (m, 20H), 2.24–2.52 (m, 8H), 3.33 ppm (br s, 2H, collapsed with D_2O).

General method for the preparation of 7-chloro-4-[(ω-hydroxyalkyl)amino]quinolines 49–53: 4,7-Dicholoroquinoline (0.6 g, 3 mmol) was mixed with the ω-hydroxyalkylamine (9 mmol) and heated in an Aldrich pressure tube at 150 °C for 3 h. After cooling, the mixture was taken up with H_2O (10 mL), and the insoluble title compound was filtered and washed with H_2O (2×5 mL) and finally crystallized from MeOH. Compounds **49–51** and **53** are already known.[43–46]

7-Chloro-4-[(2-hydroxyethyl)amino]quinoline (49): Yield: 86%; mp: 214–215 °C (lit.[43] 214 °C).

7-Chloro-4-[(3-hydroxypropyl)amino]quinoline (50): Yield: 73%; mp: 149–150 °C (lit.[44] 148–148.5 °C).

7-Chloro-4-[(4-hydroxybutyl)amino]quinoline (51): Yield: 68%; mp: 176–178 °C (lit.[45] 171–174 °C).

7-Chloro-4-[(5-hydroxypentyl)amino]quinoline (52): Yield: 82%; mp: 148–149 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42–1.98 (m, 6H, $CH_2CH_2CH_2$, + 1H, OH) 3.36 (m, 2H, NHCH_2), 3.75 (t, J = 6.0 Hz, 2H, CH_2O), 5.13 (s, 1H, NH, collapsed with D_2O), 6.42 (d, J = 5.4 Hz, 1H, arom), 7.37 (dd, J = 9.0, 2.2 Hz, 1H, arom), 7.69 (d, J = 9.0 Hz, 1H, arom), 7.98 (d, J = 2.0 Hz, 1H, arom), 8.54 ppm (d, J = 5.4 Hz, 1H, arom); elemental analysis calcd (%) for $C_{14}H_{17}ClN_2O$ (264.75): C 63.51, H 6.47, N 10.58, found: C 63.39, H 6.70, N 10.43.

7-Chloro-4-[(2-hydroxy-1-methylethy)amino]quinoline (53): Yield: 82%; mp: 207–209 °C (lit.[46] 208–210 °C).

General method for the preparation of 4-[(ω-bromoalkyl)amino]-7-chloroquinolines 53–58: An Aldrich pressure tube was sequentially charged with 7-chloro-4-(hydroxyalkylamino)quinoline (2 mmol), 48% hydrobromic acid (1.5 mL), and concentrated sulfuric acid (0.5 mL). The closed tube was heated at 160 °C for 3.5 h. After cooling, the mixture was poured on H_2O and ice and then was alkalized with 2 N NaOH solution (20 mL). The mixture was extracted with CH_2Cl_2 (3×30 mL); the organic phase was dried (Na_2SO_4) and concentrated. The residue was finally crystallized from toluene. Compounds **54**, **55**, and **58** are already known.[43,44,46]

4-[(2-Bromoethyl)amino]-7-chloroquinoline (54): Yield: 74%; mp: 139–140 °C (lit.[43] 139–140 °C).

4-[(3-Bromopropyl)amino]-7-chloroquinoline (55): Yield: 71%; mp: 158–160 °C (lit.[44] 159–160 °C).

4-[(4-Bromobutyl)amino]-7-chloroquinoline (56): Yield: 66%; mp: 220–222 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42–1.98 (m, 4H, CH_2CH_2), 3.30–3.56 (m, 2H, NHCH_2 + 2H, CH_2Br), 5.09 (s, 1H, NH, collapsed with D_2O), 6.44 (d, J = 5.4 Hz, 1H, arom), 7.38 (dd, J = 9.0, 2.0 Hz, 1H, arom), 7.71 (d, J = 9.0 Hz, 1H, arom), 8.01 (d, J = 2.0 Hz, 1H, arom), 8.59 ppm (d, J = 5.2 Hz, 1H, arom); elemental analysis calcd (%) for $C_{13}H_{14}BrClN_2$ (313.62): C 49.79, H 4.50, N 8.93, found: C 46.69, H 4.33, N 9.18.

4-[(5-Bromopentyl)amino]-7-chloroquinoline (57): Yield: 63%; mp: 112–113 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42–1.98 (m, 6H, $CH_2CH_2CH_2$), 3.29–3.58 (m, 2H, NHCH_2 + 2H, CH_2Br), 5.02 (s, 1H, NH,

collapsed with D_2O), 6.45 (d, $J=5.4$ Hz, 1H, arom), 7.40 (dd, $J=9.2$, 1.8 Hz, 1H, arom), 7.69 (d, $J=9.2$ Hz, 1H, arom), 8.00 (d, $J=1.8$ Hz, 1H, arom), 8.57 ppm (d, $J=5.4$ Hz, 1H, arom); elemental analysis calcd (%) for $C_{14}H_{16}BrClN_2$ (327.65): C 51.32, H 4.92, N 8.55, found: C 51.30, H 5.28, N 8.20.

4-[(2-Bromo-1-methylethyl)amino]-7-chloroquinoline (58): Yield: 53%; mp: 128–130 °C (lit.[46] 132–133 °C).

Biological methods

Parasite cultures and drug susceptibility assays: *P. falciparum* cultures were performed according to Trager and Jensen[54] with slight modifications. The CQ-susceptible 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% hypoxanthine, 20 mM HEPES, and 2 mM glutamine. 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 nontoxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitemia of 1–1.5% and 1% final hematocrit were aliquotted 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, according to a modified version of the method of Makler in control and drug-treated cultures.[55,56] 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.

Cytotoxicity assays: The long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV40 large T antigen was maintained in MCDB131 medium (Invitrogen, Milan, Italy) supplemented with 10% fetal calf serum (HyClone, Celbio, Milan, Italy), 10 ng mL^{-1} of epidermal growth factor (Chemicon), 1 μg mL^{-1} of hydrocortisone, 2 mM glutamine, 100 U mL^{-1} of penicillin, 100 μg mL^{-1} of streptomycin, and 20 mM HEPES buffer (EuroClone). Human Caucasian hepatocyte carcinoma (HepG2) was maintained in DMEM medium, supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U mL^{-1} of penicillin, 100 μg mL^{-1} of streptomycin, and 20 mM HEPES buffer. For the cytotoxicity assays, cells were treated with serial dilutions of test compounds for 72 h, and cell proliferation was evaluated by using the MTT assay already described.[54] The results are expressed as IC_{50}, which is the concentration of compound necessary to inhibit cell growth by 50%. Each IC_{50} value is the mean and standard deviation of least three separate experiments performed in duplicate.

Hemolysis assays: Drugs were dissolved in DMSO or medium (chloroquine) or ethanol (artemisinin) and then diluted with medium to the starting concentration of 200 μM. Compounds were placed in 96-well plates (EuroClone) and serial dilutions were made in a final volume of 100 μL well^{-1}. Fresh RBC were diluted to 1% hematocrit added to the plate and left at 37 °C for 72 h. Hemolysis was evaluated by measuring spectrophotometrically the release of hemoglobin in the supernatants (absorbance at $\lambda=405$ nm, Soret band). The percentages of hemolysis were estimated by referring to a standard curve prepared with serially diluted RBC, lysed with Triton X-100 (4%).

Keywords: antiprotozoal agents · chloroquine · cytisine derivatives · medicinal chemistry · quinolizidine derivatives

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