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Article

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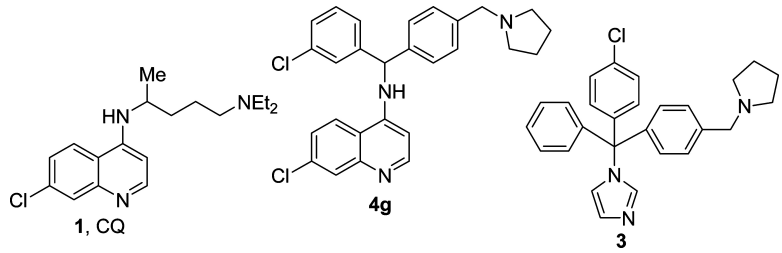

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Combining 4-Aminoquinoline- and Clotrimazole-Based Pharmacophores toward Innovative and Potent Hybrid Antimalarials

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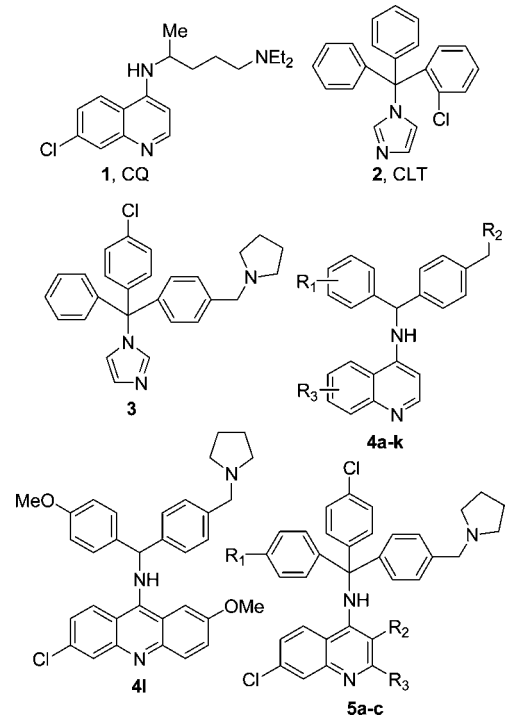
Antimalarial agents structurally based on novel pharmacophores, synthesized by low-cost synthetic procedures and characterized by low potential for developing resistance are urgently needed. Recently, we developed an innovative class of antimalarials based on a polyaromatic pharmacophore. Hybridizing the 4-aminoquinoline or the 9-aminoacridine system of known antimalarials with the clotrimazole-like pharmacophore, characterized by a polyarylmethyl group, we describe herein the development of a unique class (**4a**−**l** and **5a**−**c**) of antimalarials selectively interacting with free heme and interfering with *Plasmodium falciparum* (*Pf*) heme metabolism. Combination of the polyarylmethyl system, able to form and stabilize radical intermediates, with the iron-complexing and conjugation-mediated electron transfer properties of the 4(9)-aminoquinoline-(acridine) system led to potent antimalarials in vitro against chloroquine sensitive and resistant *Pf* strains. Among the compounds synthesized, **4g** was active in vivo against *P. chabaudi* and *P. berghei* after oral administration and, possessing promising pharmacokinetic properties, it is a candidate for further preclinical development.

Introduction

Malaria, one of the three most important diseases in Africa according to the World Health Organization, is a major cause of morbidity and mortality worldwide, especially in developing countries where it has serious economic and social costs. The disease is present in over 100 countries and threatens half of the world's population. The main reason for the recent dramatic increase in deaths from malaria is attributed to the spread of *Plasmodium falciparum* (*Pf*[a]) strains resistant to the mainstay antimalarial chloroquine (CQ, **1**, Chart 1).[1] To date, an effective therapeutic option for the treatment of resistant malaria is represented by the natural endoperoxide artemisinin and its semisynthetic derivatives, although there are concerns that artemisinin tolerant plasmodia are emerging.[2] To boost efficacy against resistant *Pf* strains, new fixed combinations are going

Chart 1. Reference (**1**−**3**) and Title (**4a**−**l**, **5a**−**c**) Compounds



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[a] Abbreviations: *Pf*, *Plasmodium falciparum*; CQ, chloroquine; ACT, artemisinin-based combination therapy; FV, food vacuole; CLT, clotrimazole; CQ-R, chloroquine-resistant; CQ-S, chloroquine-sensitive; SAR, structure-activity relationship; pLDH, parasite lactate dehydrogenase; BHIA, β-hematin inhibitory activity; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide.

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to be launched into the market that combine artemisinin derivatives with known iron protoporphirin IX-binding quinolines such as piperaquine, mefloquine, and amodiaquine (artemisinin-based combination therapies, ACTs).[2b]

Heme metabolism is involved in the antimalarial mechanism of action of both CQ and endoperoxides, and it represents a valuable therapeutic target, since its biochemistry in *Plasmodium* is entirely different from that of the host. During the intraerythrocytic stage of *Pf* life cycle, degradation of host hemoglobin occurs within the food vacuole (FV). In this site, the spontaneous oxidation of hemoglobin-derived Fe(II)-heme to Fe(III)-heme promotes the formation of superoxide ions generating H_2O_2 and hydroxyl radicals. Therefore, free heme has to be detoxified by the *Plasmodium* through highly specialized processes such as (i) dimerization of Fe(III)-heme to β-hematin and subsequent crystallization into hemozoin inside the FV and (ii) degradation by reduced glutathione in the cytoplasm.[3] Although malaria parasites live in a pro-oxidant environment, they are particularly vulnerable to oxidative stress. Consequently, the preservation of a reducing milieu (redox milieu −250 mV) through efficient redox and antioxidant systems is crucial for their survival.[4]

Our strategy for the development of innovative antimalarial pharmacophores is mainly based on the design of compounds capable of selectively interacting with free heme iron center, accumulating into the FV and being activated within its peculiar biochemical environment (pH 5.5), thus selectively producing radical species toxic for the parasites (altering *Plasmodium* fragile redox equilibria). Moreover, targeting free heme would in principle decrease the potential of a fast selection of resistant parasites, since heme is not a protein target susceptible to mutations. On the basis of these considerations, we successfully designed structurally different classes of potent antimalarials characterized by an iron-mediated mode of action.[5]

We recently described a novel class of promising clotrimazole (**2**, CLT) based antimalarials typified by **3** (Chart 1). The design strategy was based on the hypothesis that the imidazole ring, directly linked to a triarylmethyl system and endowed with appropriate electronic features (**3**), would be able to axially coordinate free heme iron leading to a conjugation-mediated electron transfer reaction inside the FV producing a putative trityl radical as the toxic species for the parasite.[5c,d] We also demonstrated that the presence of a protonatable lateral chain is critical for FV accumulation and optimal antimalarial activity. Indeed, the introduction of an extra protonatable chain in the CLT-based antimalarials was a key finding for highly improving antimalarial potency of **3** vs CLT.[5d] We also developed novel and highly potent antimalarial agents, the 4-quinolinyl- and 9-acrydinylhydrazones merging the heme interacting 4-aminoquinoline moiety of CQ with different arylhydrazone systems (these latter known to promote the metal-mediated generation of toxic radical species).[5a,e]

With the aim of generating antimalarials based on novel pharmacophores, we hybridized the structure of quinolines and acridines with the CLT-like scaffold, combining the propensity of the polyarylmethyl system of our CLT-based analogues[5c,d] to form and stabilize radical intermediates with the iron-complexing and conjugation-mediated electron transfer properties of the 4-aminoquinoline or 9-aminoacridine system of CQ and mepacrine, respectively. Accordingly, we synthesized the series of hybrids **4a**−**l** or **5a**−**c** (Chart 1 and Tables 1 and 2), presenting a simplified (benzhydryl) and the full (trityl) polyarylmethyl system of **3**, respectively. This strategy led to the development of a number of very potent antimalarials mainly active in vitro against CQ-resistant (CQ-R) parasites. The most potent analogues of the new series were selected for further in vivo pharmacological investigation. Among them, **4g** was subjected to in vivo studies using both *P. berghei* and *P. chabaudi* infected mice. Differently from **3**,[5d] compound **4g** showed good in vivo activity against both *plasmodium* species after oral administration (Table 4), and it represents a promising antimalarial agent for further preclinical development. Synthesis, molecular modeling, and biological investigation are herein discussed.

Chemistry

For the synthesis of the target compounds **4a**−**k**, two different methodologies were developed. The first approach consisted of the preparation of the properly functionalized benzhydrylamine intermediates that were coupled with the suitable 4-chloroquinolines (Scheme 1). The second approach consisted of the synthesis of the benzhydryl chloride intermediates that were reacted with the appropriate 4-aminoquinolines (Scheme 2).

Compounds **4a**−**d**,**g**−**k** were synthesized following the first approach, as described in Scheme 1. Benzophenones **6a**−**c**, prepared as previously reported,[5c] were converted into the corresponding amines **7a**−**c** through a reductive amination protocol that involved the use of ammonia in the presence of titanium(IV) isopropoxide followed by treatment with sodium borohydride (Method A in the Experimental Section). **7a-c** were obtained in yields ranging from 30% to 49%. By Exploitation of an alternative and higher yielding procedure (Method B in the Experimental Section), **7b**,**c** were also obtained by quantitative conversion of benzophenones **6b**,**c** to the corresponding oximes by using hydroxylamine hydrochloride in the presence of barium carbonate. Reduction of these latter compounds by lithium aluminum hydride afforded compounds **7b**,**c** in 72% and 65% overall yield, respectively. In the next steps of the synthetic pathway, amines **7a**−**c** were dissolved in ethoxyethanol and heated under reflux in the presence of the appropriate 4-chloroquinolines **8a**−**d**, affording the target compounds **4a**−**d**,**g**−**k**.

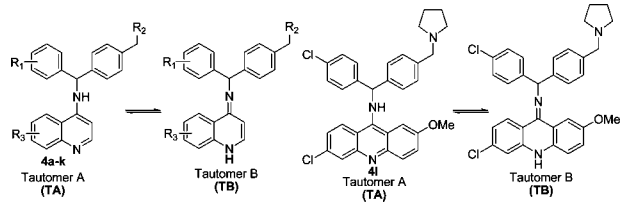
Compounds **4e** and **4f** were synthesized as depicted in Scheme 2. Ketone **6d**[5c] was reduced by sodium borohydride to afford alcohol **9**. This latter was converted into the corresponding chloride by treatment with thionyl chloride and reacted with 7-chloro- or 6-methoxy-4-aminoquinolines **11a**,**b**[6] to afford the desired compounds **4e**,**f**. Compound **4l** was prepared starting from the phenoxyacridine **12**, by treatment with amine **7a** in acetonitrile in the presence of acetic acid.

The synthesis of triarylmethyl derivatives **5a**−**c** is described in Scheme 3. Ketone **6a** was reacted with phenylmagnesium bromide or 4-chlorophenylmagnesium bromide to afford carbinols **13a**[5c] and **13b**, respectively. The tertiary alcohols were converted into the corresponding chlorides by treatment with thionyl chloride and were immediately reacted with 4-amino-7-chloroquinoline **15a** or 6-chloro-2-methoxyacridin-9-amine **15b**,[6] in the presence of triethylamine, to afford the final compounds **5a**−**c**.

Results and Discussion

1. In Vitro Antimalarial Activity and Structure−Activity Relationships (SARs). All synthesized compounds were tested in vitro as racemates against a series of *Pf* strains, namely, the CQ-sensitive (CQ-S) D10 (asynchronous culture), 3D7 (synchronous culture), and NF54 (asynchronous culture) strains and the CQ-R W2 (asynchronous culture) and K1 (either synchronous and asynchronous culture) strains. The antimalarial activity (IC_{50}, nM) was quantified as inhibition of parasite growth,

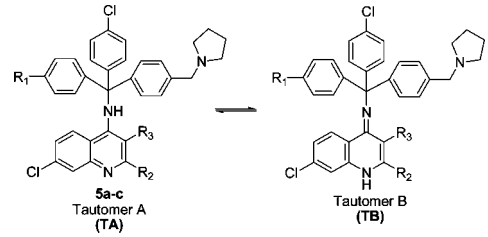
Table 1. Ionic Forms and Antiplasmodial Activity of Compounds **4a**−**l**, **3**, CLT (**2**), and CQ (**1**)



Cpd	R_1	R_2	R_3		Ionic forms (%)[a] pH 7.4	pH 7.2	pH 5.5	IC_{50} (nM)[b] D10[c]	W2[d]	3D7[c]	K1[†d]	NF54[c]	K1[‡d]
4a	4-Cl		7-Cl	TA	DP (3) P (96) N (1)	DP (5) P (95)	DP (73) P (27)	46	72	3.7	9.0	13	14
				TB	DP (71) P (29)	DP (80) P (20)	DP (99) P (1)						
4b	4-Cl		6-OMe	TA	DP (58) P (42)	DP (69) P (31)	DP (99) P (1)	57	82	8.0	60	32	32
				TB	DP (96) P (4)	DP (97) P (3)	DP (100)						
4c	4-Cl		7-CF_3	TA	DP (1) P (99)	DP (1) P (99)	DP (30) P (70)	61	90	-	30	33	20
				TB	DP (28) P (72)	DP (38) P (62)	DP (97) P (3)						
4d	4-Cl		2,8-diCF_3	TA	P (99) N (1)	P (100)	P (100)	1235	1371	100	450	-	-
				TB	DP (8) P (92)	DP (12) P (88)	DP (88) P (12)						
4e	4-Cl		7-Cl	TA	P (17) N (83)	DP (1) P (24) N (75)	DP (67) P (31) N (2)	87	84	-	-	19	21
				TB	DP (7) P (77) N (16)	DP (12) P (78) N (10)	DP (88) P (12)						
4f	4-Cl		6-OMe	TA	DP (8) P (58) N (34)	DP (13) P (63) N (24)	DP (91) P (9)	67	65	-	-	33	54
				TB	DP (9) P (89) N (2)	DP (14) P (85) N (1)	DP (89) P (11)						
4g	3-Cl		7-Cl	TA	DP (3) P (96) N (1)	DP (5) P (95)	DP (73) P (27)	21	22	24	65	12	16
				TB	DP (73) P (27)	DP (81) P (19)	DP (100)						
4h	3-Cl		6-OMe	TA	DP (58) P (42)	DP (68) P (32)	DP (99) P (1)	40	55	-	-	39	65
				TB	DP (96) P (4)	DP (97) P (3)	DP (100)						
4i	3-Cl		7-Cl	TA	P (17) N (83)	DP (1) P (24) N (75)	DP (67) P (31) N (2)	142	220	-	-	43	62
				TB	DP (7) P (77) N (16)	DP (12) P (78) N (10)	DP (88) P (12)						
4j	3-Cl		6-OMe	TA	DP (8) P (58) N (34)	DP (13) P (63) N (24)	DP (91) P (9)	86	127	-	-	43	67
				TB	DP (22) P (77) N (1)	DP (31) P (68) N (1)	DP (96) P (4)						
4k	3-Cl		7-CF_3	TA	P (15) N (85)	P (21) N (79)	DP (27) P (68) N(5)	234	304	-	-	-	-
				TB	DP (3) P (47) N (50)	DP (7) P (56) N (37)	DP (86) P (14)						
4l	-	-	-	TA	DP (17) P (83)	DP (25) P (75)	DP (94) P (6)	49	59	1.0	8.0	19	20
				TB	DP (89) P (11)	DP (93) P (7)	DP (100)						
1	-		-	TA	DP (8) P (92)	DP (12) P (88)	DP (87) P (13)	22	280	10	260	7.0	154
				TB	DP (100)	DP (100)	DP (100)						
2	-		-	-	P(5) N(95)	P(8) N(92)	P(81) N(19)	550	490	60	250	-	-
3[e]	-		-	-	DP(7) P(93) N(1)	DP(10) P(89)	DP(85) P(15)	130	59	7.0	5.0	-	-

[a] Percentage of ionic form in parentheses. DP = diprotonated form. P = protonated form. N = neutral form (ACD/pK_a DB, version 11.00, software, Advanced Chemistry Development, Inc., Toronto, Canada). [b] IC_{50} values are the mean of at least three determinations. Standard errors were all within 10% of the mean. [c] CQ-S clone. [d] CQ-R clone; K1[†], synchronous strain, K1[‡], asynchronous strain. [e] Reference 5c.

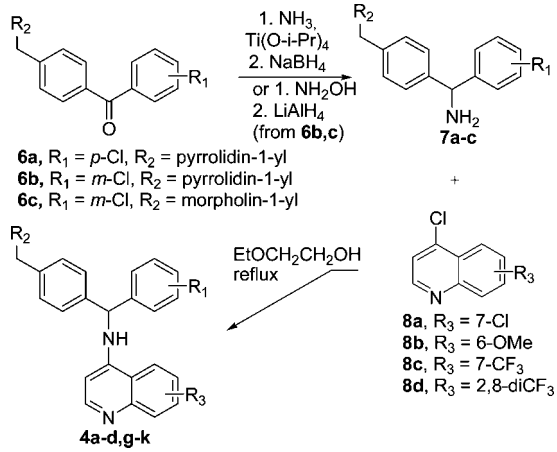
Table 2. Ionic Forms and Antiplasmodial Activity of Compounds **5a−c**, **3**, CLT (**2**), and CQ (**1**)



Cpd	R_1	R_2	R_3		Ionic forms (%)[a] pH 7.4	pH 7.2	pH 5.5	IC_{50} (nM)[b] D10[c]	W2[d]	3D7[c]	K1†[d]	NF54[c]	K1‡[d]
5a	H	H	H	TA	DP(2) P(98)	DP(3) P(97)	DP(57) P(43)	40	51	3	22	-	-
				TB	DP(64) P(36)	DP(74) P(26)	DP(99) P(1)						
5b	Cl	H	H	TA	DP(1) P(98) N(1)	DP(2) P(98)	DP(50) P(50)	48	56	-	-	16	28
				TB	DP(42) P(58)	DP(53) P(47)	DP(98) P(2)						
5c	Cl			TA	DP (7) P (93)	DP (10) P (90)	DP (85) P (15)	143	149	-	-	135	199
				TB	DP (71) P (29)	DP (80) P (20)	DP (99) P(1)						
1	-	-	-	TA	DP (8) P (92)	DP (12) P (88)	DP (87) P (13)	22	280	10	260	7.0	154
				TB	DP (100)	DP (100)	DP (100)						
2	-	-	-	-	P(5) N(95)	P(8) N(92)	P(81) N(19)	550	490	60	250	-	-
3[e]	-	-	-	-	DP(7) P(93) N(1)	DP(10) P(89)	DP(85) P(15)	130	59	7.0	5.0	-	-

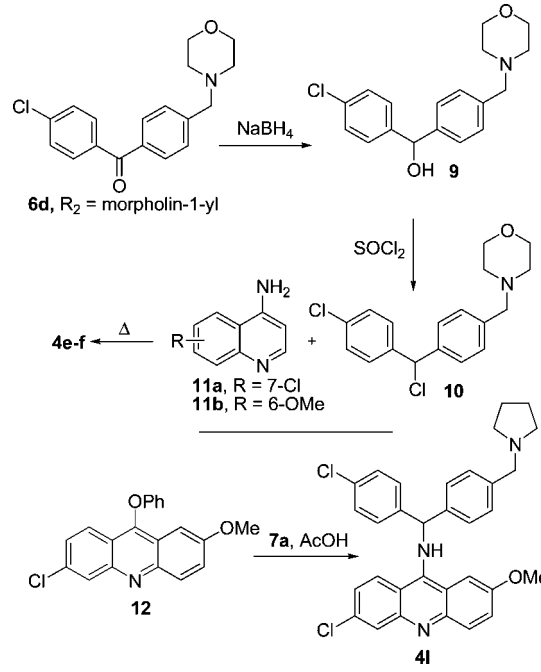
[a] Percentage of ionic form in parentheses. DP = diprotonated form. P = protonated form. N = neutral form (ACD/pK_a DB, version 11.00, software, Advanced Chemistry Development, Inc., Toronto, Canada). [b] IC_{50} values are the mean of at least three determinations. Standard errors were all within 10% of the mean. [c] CQ-S clone. [d] CQ-R clone; K1†, synchronous strain; K1‡, asynchronous strain. [e] Reference 5c.

Scheme 1. Synthesis of Compounds **4a−d**,**g−k**[a]



[a] R_{1-3} as described in Table 1.

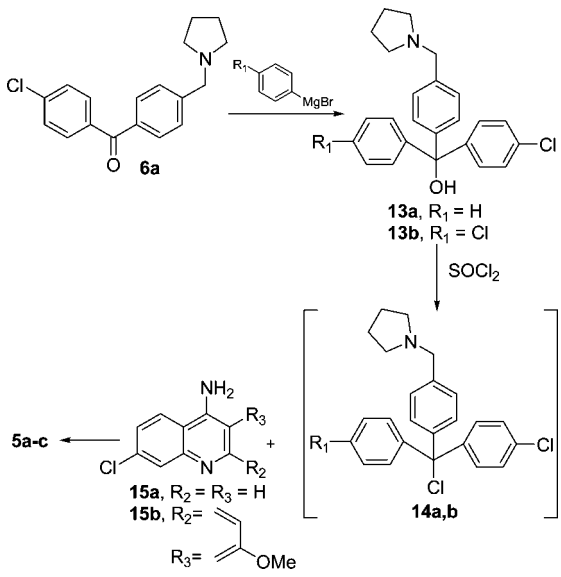
Scheme 2. Synthesis of Compounds **4e−f**,**l**



measured with the production of parasite lactate dehydrogenase (pLDH) (D10 and W2 strains) or the incorporation of [^{3}H]hypoxanthines (3D7, K1, and NF54 strains). Results are reported in Tables 1 and 2.

Following the original hypothesis of mechanism of action based on the generation of a reactive radical intermediate within the *Pf* FV,[5c,d] SARs for the novel series of antimalarials were investigated by using either the diarylmethyl (**4a−l**, Table 1) or the triarylmethyl (**5a−c**, Table 2) system as radical stabilizing groups. At the same time, we varied the number and the position of the chlorine substituents on the polyarylmethyl system and we modified the nature of the protonatable lateral chain. We also introduced specific structural modifications at the 4-aminoquinoline moiety. Indeed, the hypothesized mechanism of action of the novel antimalarials is based on the formation of an axial coordination bond between the endocyclic quinoline

Scheme 3. Synthesis of Compounds **5a**−**c**[a]



[a] R_{1-3} as described in Table 2.

nitrogen N1 and free heme iron in the specific microenvironment of the *Plasmodium* FV (as for CQ[7] and CLT derivatives[5c,d]). The electron density at N1 and the dipole moment of the quinoline ring system are likely to affect the nature of the quinoline-iron interaction, as well as the electron transfer reaction responsible for the formation of toxic radical intermediates.

(a) Effect of the Diarylmethyl Group. To fully investigate the effect on antimalarial activity of the different substituents at the 4-aminoquinoline system, we performed a comprehensive computational analysis (see Experimental Section) on all possible tautomers of compounds **4a**−**d**,**l** sharing the same disubstituted diarylmethyl-system while presenting differently substituted 4-aminoquinoline rings (Table 1). The presence of a chlorine atom at C7 of the quinoline ring (**4a**) determined (i) the prevalence of the monoprotonated form at blood and cytoplasm pH and of the diprotonated form at pH 5.5 (Table 1), optimal for FV accumulation, and (ii) a high electron density at N1 (Figure 1) with a strong molecular dipole moment, from the exocyclic nitrogen (N2) to N1 in the diprotonated form, which should enhance the ability of interacting with iron and promoting redox reactions. These electronic features, along with the calculated protonation states, made **4a** a potent antimalarial agent endowed with high activity against all *Pf* strains tested, especially against 3D7, NF54, and K1 (Table 1). Introduction of a methoxy at C6 (**4b**) and a trifluoromethyl at C7 (**4c**) slightly decreased the antimalarial potency (**4b** and **4c** vs **4a**, Table 1). The pK_a calculation showed that these substitutions affected in opposite manner the protonability of the 4-aminoquinoline moiety with respect to the chlorine at C7 (**4a**). However, in both cases (**4b** and **4c**), the different protonatability disfavored compound accumulation inside the acidic FV (Table 1), which is optimal when the compound is protonated at blood and cytoplasm pH and diprotonated at FV pH. Indeed, quantum-mechanical calculations revealed that the methoxy and the trifluoromethyl groups induced a slightly reduced electron density at N1 (Figure 1). Interestingly, we observed that the most prevalent form of **4c** (monoprotonated) presented an intramolecular hydrogen bond between the trifluoromethyl substituent and the pyrrolidine protonated nitrogen, bearing a further putative iron interaction site (Table 1, Figure 1B (bottom)). This may explain the overall good antimalarial profile of **4c** against all tested strains. The introduction of a trifluoromethyl substituent at both C2 and C8 positions of the 4-aminoquinoline scaffold resulted in a dramatic decrease of antimalarial activity against all strains (**4d** vs **4a**, **4b**, and **4c**; Table 1). The steric hindrance due to the presence of both substituents accounted for the low capability of the quinoline endocyclic N1 of interacting with the iron center of free heme, thus slashing the antimalarial potency.

These findings confirmed the significant role played by the 4-aminoquinoline ring substituents on the antimalarial activity. As a result, the introduction of the mepacrine-like system (**4l**), characterized by the favorable electronic and structural effects of the 2-chloro and the 6-methoxy substituents, allowed us to obtain a potent antimalarial against all *Pf* strains tested (**4l** vs **4a**, **4b**, and **4c**; Table 1; Figure 1). In particular, pK_a calculations and quantum mechanical studies revealed some unique features of **4l** such as (i) the presence of a further putative iron interaction site between the methoxy and pyrrolidine groups in the protonated form (prevalent at cytoplasm pH, Table 1), (ii) an increased electron density at N1 with respect to **4b** and **4c**, in the protonated form, (iii) the highest electron density at N1 of the whole series, in the diprotonated form (prevalent at FV pH, Table 1), and (iv) the presence of a strong molecular dipole moment between N2 and N1 in both protonated and diprotonated forms. These electronic features, in agreement with the hypothesized mechanism of action, explain the potent activity of **4l** (Table 1).

According to our previous results on the CLT-based class of antimalarials,[5c,d] we maintained the *p*- or *m*-Cl substituent on the diarylmethyl system (Table 1), since, being an electron-withdrawing group, it plays a key role in the formation and stabilization of the putative radical species. Interestingly, global minimum conformers of **4a**−**l** evidenced a network of intramolecular interactions between polarized hydrogens at C2 of both phenyl rings of the diphenylmethyl system and the N2(H) group (distance of ∼2.8 Å) in both the protonated and diprotonated forms. As revealed by a fragment based search in the Cambridge Structural Database (CSD) (Conquest 1.9, CSDS) and depicted in Figure 2 of the main text and Figure 1 of the Supporting Information for compounds **4g** and **4a**, this introduced a further putative iron interaction site in the structure. Moreover, in the most stable conformers, the H-bond properties of the N2(H) group allowed the stabilization of both 4-aminoquinoline tautomers without affecting the tautomeric equilibria. It is well-known that the existence of two tautomeric forms accounts for the peculiar physical−chemical properties of 4-aminoquinolines, such as (i) the increased electron density at N1 (dipole moment), (ii) the consequent higher degree of protonatability with respect to 4-unsubstituted quinolines, and (iii) their chemical reactivity.[8] Compound **4g** resulted one of the most potent compounds of the present series against the asynchronous strains D10 and W2 and was demonstrated to a have a comparable activity against all *Pf* strains tested.

The nature of the protonatable lateral chain can modulate antimalarial activity affecting the pK_a value of the compound and, consequently, FV accumulation. In order to explore this SAR, we introduced either the *p*-pyrrolidinylmethyl moiety of **3** (**4a**−**d** and **4g**,**h**; Table 1) or a *N*-morpholinemethyl (**4e**,**f**,**i**−**k**; Table 1) as protonatable lateral chain. Although in general the morpholine ring is less protonatable than the pyrrolidine ring, the prevalent ionic form of the compound at pH 7.2/7.4 and 5.5 is also dependent upon the nature and the position of the substituents on the quinoline (R_3, Table 1). In particular, in the 4-aminoquinoline series, the capability of the substituents

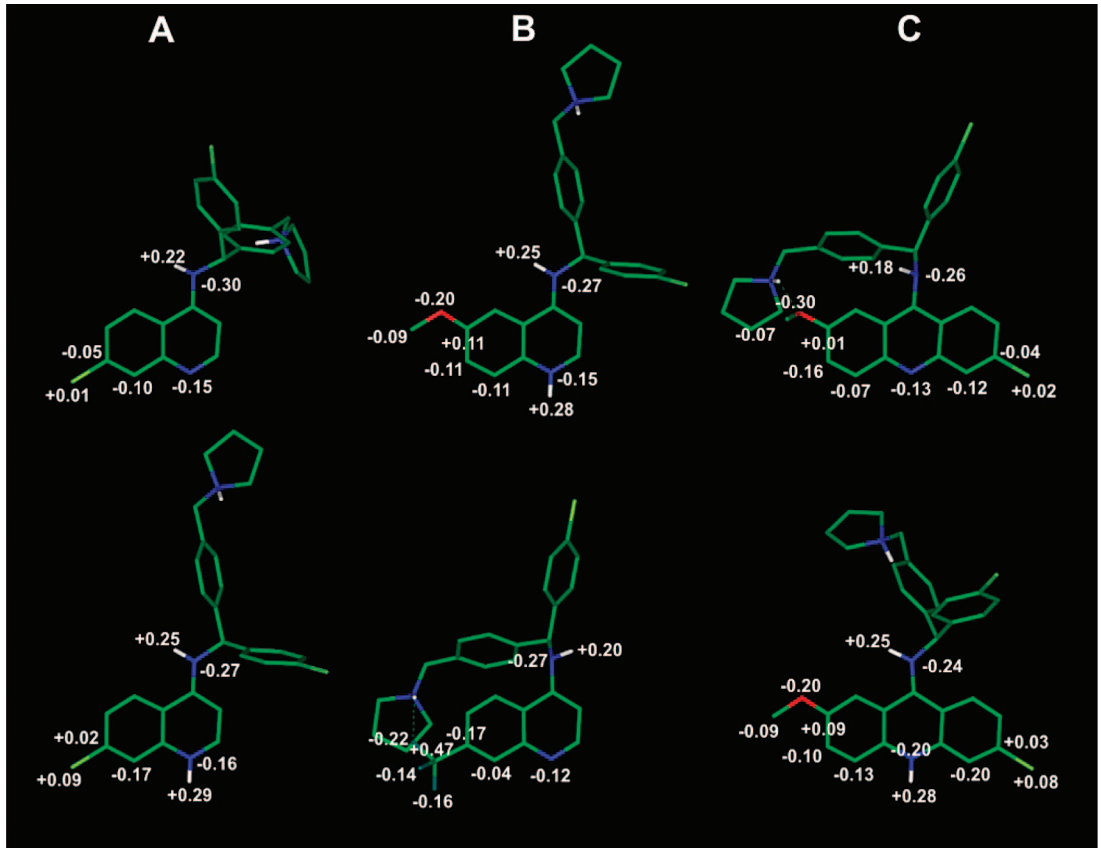


Figure 1. Global minimum conformers of **4a** (A), **4b** (B, top), **4c** (B, bottom), and **4l** (C) in their prevalent ionic form(s) at cytoplasm and FV pH according to calculated pK_a values reported in Table 1. The compounds are colored by atom type. All hydrogens, except those of the protonable nitrogens, have been omitted for clarity. MOPAC (AM1) partial atomic charges are displayed as white labels.

introduced (R_3) of improving the protonability of the nitrogen of the lateral chain showed the following rank order: 6-OMe > 7-Cl > 7-CF_3 (Table 1). According to the hypothesized mechanism of action, the pharmacological results on all *Pf* strains tested confirmed that potency was dependent upon the different capability of R_3 substituents of affecting the protonability of the 4-aminoquinoline moiety. These findings demonstrated the importance of a fine-tuning of the electronic properties in order to optimally balance the tropism for the FV and accumulation.

(b) Triarylmethyl versus Diarylmethyl Derivatives. Similarly to compound **3**, the analogues **5a**−**c** (Table 2) are characterized by the presence of the trityl system coupled to the fused 4(9)-aminoquinoline(acridine). In contrast to CLT derivatives,[5c,d] the introduction of the triarylmethyl group, either on the 4-aminoquinoline system of **5a** and **5b** or on the mepacrine-like system of **5c**, negatively affects the protonatability of the compounds at the considered pH values (Table 2). Consequently, in this new series of hybrid compounds, the favorable effect of the trityl system on the propensity to form and stabilize the putative radical intermediates observed in the original series of CLT analogues[5c,d] is "counterbalanced" by the reduced pK_a value of the 4-aminoquinoline moieties of **5a** and **5b** and of the mepacrine-like system of **5c**, crucial for the double protonation at the FV pH (Table 2). This latter property, as already outlined, is likely to affect both the accumulation inside the acidic FV (pharmacokinetic properties) and the nature of the interaction with heme iron (pharmacodynamic properties). Accordingly, the trityl derivatives **5a**, **5b**, and **5c** did not show a highly improved antimalarial activity over the corresponding benzhydryl analogues **4a**, **4b**, and **4l** (Tables 1 and 2).

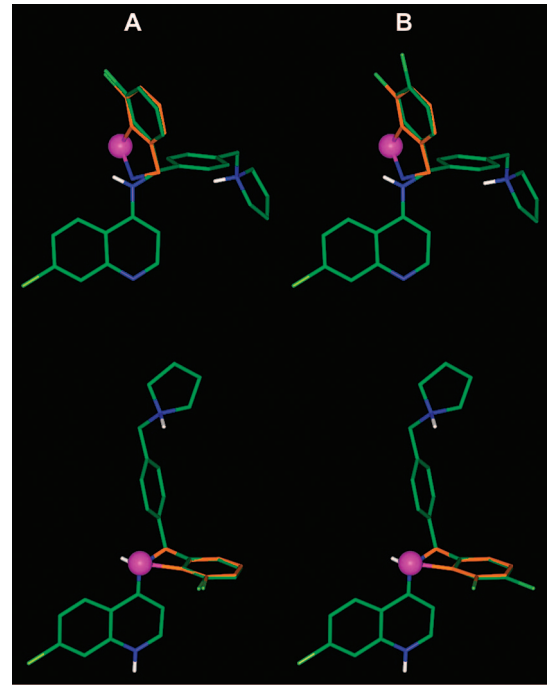


Figure 2. Global minimum conformers of **4g** (A) and **4a** (B) in their prevalent ionic forms at cytoplasm (top) and FV (bottom) pH according to calculated pK_a values reported in Table 1. The superimposition on the X-ray structure of an iron complex containing the (3-chlorophenyl)methanamine moieties (orange, CSD code ATIGAL) is reported. Iron vdW volume (magenta) are scaled for clarity of presentation. Atoms are colored by atom type: H, white; C, green; N, blue; Fe, magenta; Cl, light-green.

Table 3. In Vitro Cellular Toxicity of Compounds **4a**−**d**,**g**,**l**, **5a**, **3**, CLT (**2**), and CQ (**1**)

	TC_{50} (μM)[a]			
compd	NSO cells[b]	Daudi cells[c]	normal human lymphocytes[d]	KB cell[e]
4a	8.6	8.6	17.3	19.6
4b	nt	nt	nt	8.5
4c	12.1	14.1	24.2	5.5
4d	nt	nt	nt	213
4g	23.8	34.6	54.1	nt
4l	11.1	11.1	16.6	nt
5a	14.9	16.7	18.6	nt
3	51.5	58.5	70.3	nt
2, CLT	nt	nt	nt	88.1
1, CQ	nt	nt	nt	207.0

[a] Standard errors never exceeded 5% of the reported values. nt = not tested. [b] Plasmocytoma murine cell line. [c] Human lymphoblastoid cell line. [d] Normal human lymphocytes PHA-stimulated. [e] Human carcinoma of the nasopharynx cell line.

On the bases of the in vitro results against CQ-S and CQ-R strains, a selected set of compounds was subjected to further biological evaluation.

2. Cytotoxicity: MTT Assays. Cytotoxicity for compounds **4a**−**d**,**g**,**l** and **5a** (Table 3) was evaluated using plasmocytoma murine cell line (NSO cells), normal human lymphocytes PHA-stimulated, and human lymphoblastoid cell line (Daudi cells). On KB cells (a cell line derived from a human carcinoma of the nasopharynx) the cytotoxicity of selected compounds was compared to the reference antimalarials CQ and CLT. As displayed in Table 3, all compounds tested revealed a low in vitro toxicity against the different human and murine cell lines, although on KB cells, all compounds tested, except **4d**, proved to be slightly more cytotoxic than the reference compounds.

3. In Vivo Antimalarial Activity. Preliminary evaluation of the in vivo antimalarial activity of compounds **4a**,**c**,**g**,**l** and **5a** was performed in female CD1, 20−25 g mice infected with *P. chabaudi* AS (Table 4). **4g** was also tested in mice infected by *P. berghei* ANKA. Activity of **2** and **3** against *P. berghei* and *P. chabaudi* was previously reported.[5d] For both murine strains of *Plasmodium,* in vivo antimalarial activity evaluation was performed according to the four-day suppressive test of Peters et al.[9] Compounds were administered once daily for 4 days, with the first drug administered 2 h after iv parasite inoculation. All compounds tested (**4a**,**c**,**g**,**l** and **5a**) exerted a 98.0−99.9% parasitemia suppression after oral dosing (4 × 50 mg/kg) (Table 4) against *P. chabaudi*, a *Plasmodium* strain able to produce synchronous infections in mice. Since **4g** presented a similar potency against both synchronous and asynchronous strains in vitro, it was also tested in mice infected by *P. berghei*, which induces an asynchronous infection. When administered once daily for 4 days at a dose of 50 mg/kg, **4g** induced a 98.0% (±3.30) suppression of parasitemia. The 50% efficient dose (ED_{50}) of **4g** was evaluated from a plot of activity (expressed as a percentage of the control) versus the dose (orally administered once daily for 4 days) and resulted in 6.3 mg/kg against *P. berghei.* Therefore, this molecule represents a promising lead compound for the development of a novel class of highly potent antimalarials able to overcome CQ resistance.

4. β-Hematin Inhibitory Activity Assay. 4g, one of the most potent compounds in vitro and in vivo, was screened for inhibition of β-hematin formation by using the β-hematin inhibitory activity (BHIA) assay[10] in order to confirm its mechanism of action based on the interference with the heme detoxification process of *Pf*. Compound **4g** showed a dose-dependent inhibition of β-hematin formation (Table 5) with an IC_{50} lower than those of the reference compounds, thus demonstrating a better ability to interact with iron-heme.

5. Preliminary Pharmacokinetic Studies. Encouraged by these promising in vitro and in vivo antimalarial data, we started preliminary disposition studies of compounds **4a**,**g** in mice and rats. After intravenous injection of **4a** (5 mg/kg) in the rat, the concentrations in plasma rapidly fell below the limit of quantification of the analytical procedure within 60 min of dosing (Figure 3). The apparent volume of distribution was large (40 ± 20 L/kg), but body clearance was high, explaining the short elimination $t_{1/2}$ of this compound in this species (44 ± 35 min). Under the same experimental conditions, **4g** was distributed in a similar volume as **4a** (48 ± 13.3 L/kg) but was cleared slowly compared to its structurally related compound, displaying an elimination $t_{1/2}$ of about 3 h with a biexponential decay of the plasma concentration. The blood-to-plasma ratio for compound **4g** averaged 1.1 ± 0.1 suggesting a uniform distribution throughout the rat blood, with limited plasma protein binding. After oral doses of **4g** in rat, at least three putative metabolite peaks were observed in the plasma chromatograms, suggestive of a possible extensive first-pass biotransformation. This compound rapidly reached the systemic circulation achieving maximal plasma concentrations (C_{max}) within 60 min after administration of 50 mg/kg free base. A similar behavior was detected in mice, although mean C_{max} (0.33 ± 0.12 μg/mL) and AUC_t (43.5 μg/(mL·min)) were higher than in rats (0.18 ± 0.11 μg/mL and 25.3 μg/(mL·min), respectively). This may be due to differences in the rate and extent of absorption from the gastrointestinal tract and, more likely, to first-pass metabolism which would include the formation of the three putative metabolites found in plasma of both species. Structural characterization of the three metabolites is currently in progress. Findings were similar in liver where **4g** achieved concentrations several times those in plasma, indicating preferential tropism of **4g** for this tissue. At the first time, 15 min after administration, **4g** liver concentrations were already much higher than in blood in both species (Figure 4). At 180 min, **4g** concentrations still increased, averaging 52.2 and 30.5 μg/g in the mouse and rat, respectively. Mean liver AUC_t was 7061 μg/g in the mouse and 2945 μg/g in the rat, yielding a mean liver-to-plasma distribution ratio above 100 in both species.

Conclusions

In summary, a novel polycyclic hybrid pharmacophore was exploited to design potent antimalarials against CQ-R strains. Combination of the polyarylmethyl system of clotrimazole-based analogues (able to form and stabilize radical intermediates) with the 4-aminoquinoline system (endowed with iron-complexing and conjugation-mediated electron transfer properties) resulted in the discovery of a novel series of potent antimalarials. By means of our design strategy, we identified extremely potent CQ/CLT hybrids particularly active against CQ-R *Pf* strains and able to reduce parasitemia after oral administration. Starting from **3**, a novel antimalarial agent active in vivo against infections produced by *P. chabaudi* but not by *P. berghei* (displaying a narrow spectrum of activity),[5d] the hybridization process herein described led to the development of **4g**, a potent lead compound in both in vitro and in vivo tests. Compound **4g**, characterized by unique pharmacophoric features, showed potent antimalarial activity in vitro mainly against CQ-R strains. Moreover, after oral administration in vivo, it displayed a broader spectrum of activity, being active against both *P. berghei* and *P. chabaudi* infections. Furthermore, we proved for **4g** promising preliminary pharmacokinetic properties. Being struc-

Table 4. In Vivo Antimalarial Activity of Compounds **4a**,**c**,**g**,**l**, **5a**, **3**, CLT (**2**), and CQ (**1**) against *P. berghei* and *P. chabaudi* (4 Days Peters' Test) after Oral Administration[a]

		P. berghei ANKA	
compd	*P. chabaudi* AS, % suppression on day 4 ± SD[b] (mg/kg)	% suppression on day 4 ± SD[b] (mg/kg)	survival, no. of days after infection[c]
4a	99.5 ± 0.14 (50)	nt	
4c	99.6 ± 0.10 (50)	nt	
4g	98.0 ± 0.80 (50)	98.0 ± 3.30 (50)	18
4l	99.9 ± 0.05 (50)	nt	
5a	99.9 ± 0.05 (50)	nt	
3[d]	94.8 ± 4.29 (50)	37.6 ± 1.98 (30)[e]	
1,[d] CQ	99.9 ± 0.02 (10)	99.2 ± 0.56 (10)	16.3
2,[d] CLT	6.0 ± 3.48 (50)	28.3 ± 2.52 (150)	

[a] In vivo tests were performed under the UK Home Office Animals (Scientific Procedures) Act 1986. [b] Percent suppression = $[(C - T)/C] \times 100$; where C = parasitemia in control group and T = parasitemia in treated group (nt, not tested) after oral administration; SD = standard deviation. Five mice per group were used. [c] All mice that survived 18 days were sacrificed; survival of vehicle treated controls was 10.0 days. [d] Reference 5d. [e] Intraperitoneal administration.

Table 5. β-Hematin Inhibitory Activity Assay of Compounds **4g**, **3**, CLT (**2**), and CQ (**1**)

compd	IC_{50}[a]
4g	1.33
3	2.50
2, CLT	2.53
1, CQ	1.69

[a] The IC_{50} represents the molar equivalents of compound, relative to hemin, that inhibit β-hematin formation by 50%. Data are the mean of three different experiments in triplicate. Standard errors were all within 10% of the mean.

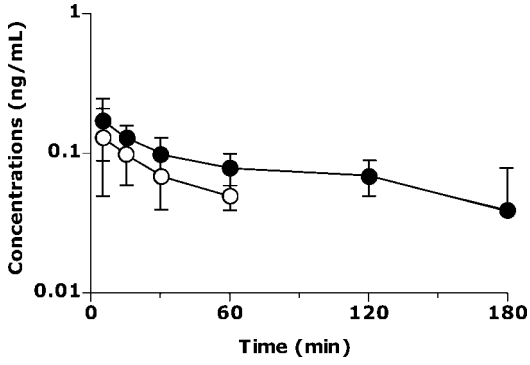


Figure 3. Plasma concentration−time curves of **4a** and **4g** after intravenous injection of their hydrochloride salt in rats. Each value is the mean (±SD) of three animals for compound **4a** (open circles) and **4g** (closed circles). The two compounds were injected at a dose of 5 mg/kg, in water for injection.

turally related to CLT-derived antimalarials, compound **4g** was also tested against *Candida* spp. and *Aspergillus* species, demonstrating no antimycotic activity (MIC > 32 μg/mL) because of a highly reduced capability of interacting with the cytochrome 14α-lanosterol demethylase. Finally, when tested on human and murine cell lines, **4g** showed low in vitro cytotoxicity. On the basis of these pharmacological properties, compound **4g** was selected as a candidate for further preclinical investigation.

Experimental Section

Chemistry. Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F254 (0.040−0.063 mm) with detection by UV. Merck silica gel 60 (0.040−0.063 mm) was used for column chromatography. Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 200 MHz or Varian 300 MHz spectrometers with TMS as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (J) in hertz (Hz). ESI-MS spectra were performed by an Agilent 1100 series LC/MSD spectrometer. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer, and the results were within ±0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. Intermediates **6a**,**b**,**d**, **13a**,[5c,d] and quinolines **11a**,**b** and **15a**,**b**[6] were prepared as previously described. Hydrochloride salts of analogues for biological investigation were prepared following standard procedures.

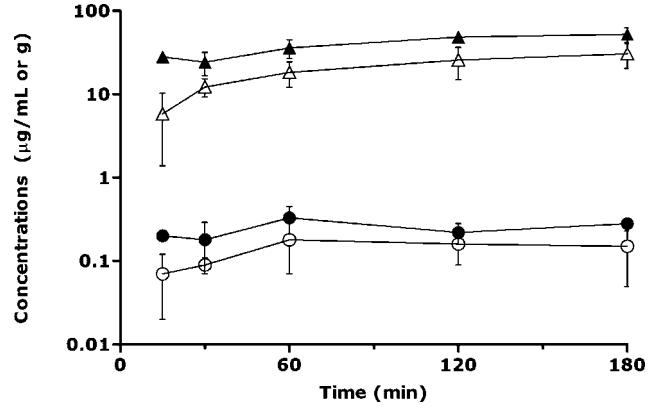


Figure 4. Plasma and liver concentration−time curves of compounds **4g** after oral administration in the mouse and rat. The dose of compound **4g** was 50 mg/kg (po). Each value is the mean (±SD) of three animals for plasma (circles) and liver (triangles) in mice (closed symbols) and rats (open symbols).

(3-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methanone (6c). To a stirred solution of [4-(bromomethyl)phenyl](3-chlorophenyl)methanone[5c] (3.5 g, 11.3 mmol) in dry MeCN (75 mL), cooled to 0 °C, morpholine (1.47 mL, 16.9 mmol) and triethylamine (3.1 mL, 22.6 mmol) were added, and the resulting mixture was allowed to stir at 0 °C for 3 h. Thereafter, the reaction was quenched with H_2O and the solvent was evaporated under reduced pressure. The residue was treated with H_2O and extracted with $CHCl_3$ (2 × 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash-chromatography (1:20 MeOH/$CHCl_3$) to afford **6c** (3.2 g, 75%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67−7.72 (m, 3H), 7.67−7.63 (m, 1H), 7.56−7.52 (m, 1H), 7.48−7.38 (m, 3H), 3.73−3.70 (m, 4H), 3.57 (s, 2H), 2.48−2.45 (m, 4H); ESI-MS m/z $(M + H)^+$ 316.

(±)-(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methanamine (7a). To a mixture of **6a** (2.8 g, 9.4 mmol) and 2.0 M solution of ammonia in EtOH (24 mL, 50 mmol), titanium(IV) isopropoxide (5.5 mL, 18.8 mmol) was added, and the reaction

mixture was allowed to stir at 25 °C for 16 h. Subsequently, sodium borohydride (0.53 g, 14.0 mmol) was added and the mixture was stirred at 25 °C for further 3 h, then poured into a 2.0 M solution of ammonium hydroxide. The inorganic precipitate was filtered off, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were washed with brine and dried over Na_2SO_4, and the solvent was removed. The residue was purified by flash chromatography (5% MeOH in $CHCl_3$) to afford 0.83 g (30%) of **7a** as colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40−7.36 (m, 1H), 7.28−7.21 (m, 4H), 7.21−7.17 (m, 3H), 5.14 (s, 1H), 3.61 (s, 2H), 2.55−2.53 (m, 4H), 2.28 (bs, 2H), 1.88−1.85 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 301.

(±)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methanamine (7b). Method A. Starting from **6b**, the title compound was prepared following the procedure described for the synthesis of **7a** and was obtained as a yellow thick oil (35%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34−7.22 (m, 8H), 5.15 (s, 1H), 3.58 (s, 2H), 2.54 (m, 4H), 2.0 (bs, 2H), 1.81 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 301.

Method B. To a solution of **6b** (1.2 g, 4 mmol) in MeOH (50 mL), hydroxylamine hydrochloride (641 mg, 9.2 mmol) and barium carbonate (1.82 g, 9.2 mmol) were added, and the mixture was heated under reflux. After 18 h, the reaction mixture was cooled to 25 °C, the inorganic precipitate was filtered off, and the solvent was removed under reduced pressure. The oxime thus obtained was dissolved in anhydrous tetrahydrofuran (THF) (15 mL), and the resulting solution was added dropwise to a suspension of lithium aluminum hydride (485 mg, 12.8 mmol) in THF (20 mL). The mixture was heated under reflux for 1 h. Afterward the excess of lithium aluminum hydride was quenched by the addition of EtOH and H_2O. The inorganic precipitate was filtered off, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography, eluting with 5% MeOH in $CHCl_3$ to afford **7b** (0.86 g) in 72% yield.

(±)-(3-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methanamine (7c). Starting from **6c**, the title compound was prepared following method A or B and was obtained as a yellow thick oil in 49% and 65% yields, respectively: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.11 (m, 8H), 5.07 (s, 1H), 3.62−3.59 (m, 4H), 3.39 (s, 2H), 2.36−2.33 (m, 4H), 2.04 (bs, 2H); ESI-MS *m*/*z* $(M + H)^+$ 317.

(±)-(4-Chlorophenyl)[4-(morpholinomethyl)phenyl]methanol (9). To a solution of **6d** (580 mg, 1.84 mmol) in EtOH (30 mL), sodium borohydride (105 mg, 2.76 mmol) was added, and the resulting mixture was stirred at 25 °C for 30 min. The solvent was then removed under reduced pressure, the residue was taken up in a saturated solution of ammonium chloride and the aqueous layer was extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give **9** (0.51 g, 87%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25−7.23 (m, 8H), 5.67 (s, 1H), 4.03 (bs, 1H), 3.57 (m, 4H), 3.41 (s, 2H), 3.38 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 318.

Bis(4-chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methanol (13b). A solution of **6a** (1.1 g, 3.8 mmol) in anhydrous THF (25 mL) was added dropwise to 4-chlorophenylmagnesium bromide (1.0 M in THF, 3.8 mL, 3.8 mmol), and the resulting solution was heated to 75 °C for 8 h. The reaction mixture was quenched with 25 mL of 20% of ammonium chloride solution. The aqueous layer was extracted with EtOAc (2 × 25 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (2% MeOH in $CHCl_3$) to give **13b** (1.0 g, 66%) as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26−7.11 (m, 12H), 3.58 (s, 2H), 2.49 (m, 4H), 1.73 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 412.

(±)-*N*-{(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (4a). To a solution of **7a** (0.83 g, 2.8 mmol) in 2-ethoxyethanol (10 mL), 4,7-dichloroquinoline **8a** (0.57 g, 2.9 mmol) and pyridine hydrochloride (0.35 g, 3.0 mmol) were added. The resulting mixture was heated to 135 °C for 5 h. Afterward it was cooled to 25 °C. Triethylamine (0.5 mL) was added, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (5% MeOH in $CHCl_3$) to afford **4a** (0.91 g) in 70% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (d, 1H, J = 5.3 Hz), 7.97 (d, 1H, J = 2.0 Hz), 7.72 (d, 1H, J = 8.8 Hz), 7.40−7.26 (m, 9H), 6.23 (d, 1H, J = 5.3 Hz), 5.71 (d, 1H, J = 4.1 Hz), 5.41 (d, 1H, J = 4.1 Hz), 3.63 (s, 2H), 2.55 (m, 4H), 1.80 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.2, 149.3, 148.5, 140.1, 139.6, 139.4, 130.1, 129.9, 129.4, 129.3, 129.2, 128.9, 127.6, 125.9, 121.2, 117.4, 101.5, 61.7, 60.5, 54.5, 23.7; ESI-MS *m*/*z* $(M + H)^+$ 462. Anal. ($C_{27}H_{25}Cl_2N_3$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-6-methoxy-4-aminoquinoline (4b). Starting from **7a** and 6-methoxy-4-chloroquinoline **8b**, the title compound was prepared following the above-described procedure: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.31 (d, 1H, J = 5.0 Hz), 7.93 (d, 1H, J = 9.1 Hz), 7.38−7.26 (m, 8H), 7.03 (d, 1H, J = 2.6 Hz), 6.22 (d, 1H, J = 5.3 Hz), 5.71 (d, 1H, J = 4.1 Hz), 5.32 (d, 1H, J = 4.1 Hz), 3.86 (s, 3H), 3.60 (s, 2H), 2.51 (m, 4H), 1.78 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.2, 148.8, 147.6, 144.3, 140.0, 139.9, 139.8, 133.7, 131.9, 129.9, 129.3, 129.0, 127.7, 120.5, 119.6, 101.6, 99.4, 61.8, 60.5, 56.0, 54.6, 23.7; ESI-MS *m*/*z* $(M + H)^+$ 458. Anal. ($C_{28}H_{28}ClN_3O$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-7-trifluoromethyl-4-aminoquinoline (4c). Starting from **7a** and 7-trifluoromethyl-4-chloroquinoline **8c**, the title compound was prepared following the above-described procedure: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 5.3 Hz), 8.29 (s, 1H), 7.91 (d, 1H, J = 8.8 Hz), 7.61 (dd, 1H, J = 1.8, 8.8 Hz), 7.39−7.26 (m, 8H), 6.33 (d, 1H, J = 4.4 Hz), 5.72 (d, 1H, J = 4.4 Hz), 5.48 (d, 1H, J = 4.4 Hz), 3.65 (s, 2H), 2.56 (m, 4H), 1.82 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 496. Anal. ($C_{28}H_{25}ClF_3N_3$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(pyrrolidin-1ylmethyl)phenyl]methyl}-2,8-di(trifluoromethyl)-4-aminoquinoline (4d). Starting from **7a** and 2,8-di(trifluoromethyl)-4-chloroquinoline **8d**, the title compound was prepared following the above-described procedure: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10 (d, 1H, J = 5.6 Hz), 8.02 (d, 1H, J = 7.3 Hz), 7.59 (m, 1H), 7.43−7.26 (m, 8H), 6.62 (d, 1H, J = 2.9 Hz), 5.78 (s, 1H), 5.67 (s, 1H), 3.70 (s, 2H), 2.61 (m, 4H), 1.84 (m, 4H); ESI-MS *m*/*z* $(M + H)^+$ 564. Anal. ($C_{29}H_{24}ClF_6N_3$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (4e). To a solution of **9** (0.25 g, 0.78 mmol) in dry CH_2Cl_2 (8 mL), cooled to 0 °C, a solution of $SOCl_2$ (451 μL, 6.18 mmol) in dry CH_2Cl_2 (4 mL) was added, and the resulting mixture was stirred at 0 °C for 20 min and at 45 °C for 4 h. The volatiles were removed and the residue was treated with dry MeCN (3 × 4 mL) and concentrated under reduced pressure in order to remove residual $SOCl_2$. The resulting hydrochloride salt was suspended in dry MeCN (12 mL), and to this solution was added a solution containing triethylamine (430 μL, 3.0 mmol) and 4-amino-7-chloroquinoline **11a** (0.16 g, 0.94 mmol) at 0 °C. Thereafter, the reaction mixture was heated to 80 °C for 6 h. The solvent was evaporated under reduced pressure, and the residue was treated with H_2O and extracted with EtOAc (4 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude residue was purified by flash column chromatography (2% MeOH in $CHCl_3$) to afford **4e** as a pale-yellow amorphous solid (0.11 g, 32%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, 1H, J = 5.2 Hz), 7.98 (s, 1H), 7.71 (d, 1H, J = 9.0 Hz), 7.39−7.26 (m, 9H), 6.23 (d, 1H, J = 5.2 Hz), 5.70 (d, 1H, J = 4.1 Hz), 5.38 (d, 1H, J = 3.8 Hz), 3.70 (m, 4H), 3.49 (s, 2H), 2.44 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.2, 149.3, 148.5, 139.8, 139.5, 138.4, 135.2, 134.0, 130.2, 129.5, 129.2, 128.9, 127.6, 125.9, 121.2, 117.4, 101.5, 67.2, 63.2, 61.7, 53.8; ESI-MS *m*/*z* $(M + H)^+$ 478. Anal. ($C_{27}H_{25}Cl_2N_3O$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methyl}-6-methoxy-4-aminoquinoline (4f). Starting from **9** (0.25 g, 0.78 mmol) and 6-methoxy-4-aminoquinoline **11b**, the title compound was prepared following the above-described procedure and was obtained as a brown oil (14%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, 1H, J = 5.2 Hz), 7.91 (d, 1H, J = 9.3 Hz), 7.34−7.26 (m, 9H), 7.03 (s, 1H), 6.23 (d, 1H, J = 5.2 Hz), 5.71 (d, 1H, J = 3.8 Hz), 5.28 (d, 1H, J = 3.5 Hz), 3.85 (s, 3H), 3.70 (m, 4H), 3.49 (s, 2H), 2.44 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.2, 148.7,

147.6, 144.1, 140.1, 139.9, 138.3, 133.9, 131.8, 130.1, 129.4, 128.9, 127.7, 120.3, 119.6, 101.6, 99.6, 67.2, 63.2, 61.8, 56.0, 53.9; ESI-MS *m/z* (M + H)$^+$ 474. Anal. ($C_{28}H_{28}ClN_3O_2$) C, H, N.

(±)-*N*-{(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (4g). Starting from **7b** and **8a**, the title compound was prepared following the procedure described for the synthesis of **4a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.44 (d, 1H, J = 5.3 Hz), 7.98 (d, 1H, J = 2.0 Hz), 7.72 (d, 1H, J = 8.8 Hz), 7.41−7.26 (m, 9H), 6.23 (d, 1H, J = 5.3 Hz), 5.68 (d, 1H, J = 4.4 Hz), 5.39 (d, 1H, J = 4.4 Hz), 3.68 (s, 2H), 2.60 (m, 4H), 1.83 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.2, 149.3, 148.4, 143.2, 140.2, 139.3, 135.2, 130.6, 130.0 (2C), 129.3, 128.4, 127.7, 127.6, 125.9, 125.6, 121.1, 117.4, 101.5, 61.9, 60.5, 54.6, 23.7; ESI-MS *m/z* (M + H)$^+$ 462. Anal. ($C_{27}H_{25}Cl_2N_3$) C, H, N.

(±)-*N*-{(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-6-methoxy-4-aminoquinoline (4h). Starting from **7b** and **8b**, the title compound was prepared following the above-described procedure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, 1H, J = 5.3 Hz), 7.91 (d, 1H, J = 9.4 Hz), 7.36−7.25 (m, 9H), 7.08 (s, 1H), 6.22 (d, 1H, J = 5.3 Hz), 5.70 (d, 1H, J = 4.1 Hz), 5.47 (d, 1H, J = 3.8 Hz), 3.85 (s, 3H), 3.62 (s, 2H), 2.55 (m, 4H), 1.79 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.2, 148.9, 147.5, 144.3, 143.6, 139.9, 139.7, 135.1, 131.9, 130.5, 129.9, 128.3, 127.7, 127.6, 125.7, 120.4, 119.6, 101.6, 99.4, 62.0, 60.5, 56.0, 54.6, 23.7; ESI-MS *m/z* (M + H)$^+$ 458. Anal. ($C_{28}H_{28}ClN_3O$) C, H, N.

(±)-*N*-{(3-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (4i). Starting from **7c** and **8a**, the title compound was prepared following the above-described procedure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, 1H, J = 5.3 Hz), 8.0 (s, 1H), 7.72 (d, 1H, J = 9.1 Hz), 7.41−7.26 (m, 9H), 6.25 (d, 1H, J = 5.6 Hz), 5.70 (d, 1H, J = 4.1 Hz), 5.41 (d, 1H, J = 3.2 Hz), 3.72−3.70 (m, 4H), 3.50 (s, 2H), 2.47−2.45 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.9, 149.0, 148.6, 143.0, 139.5, 138.5, 135.4, 135.3, 130.6, 130.2, 129.0, 128.5, 127.7, 127.6, 126.0, 125.6, 121.2, 117.3, 101.5, 67.2, 63.2, 61.9, 53.8; ESI-MS *m/z* (M + H)$^+$ 478. Anal. ($C_{27}H_{25}Cl_2N_3O$) C, H, N.

(±)-*N*-{(3-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]methyl}-6-methoxy-4-aminoquinoline (4j). Starting from **7c** and **8b**, the title compound was prepared following the above-described procedure and was obtained as a white amorphous solid (40%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, 1H, J = 5.0 Hz), 7.94 (d, 1H, J = 9.4 Hz), 7.36−7.26 (m, 9H), 7.02 (s, 1H), 6.23 (d, 1H, J = 5.0 Hz), 5.69 (d, 1H, J = 3.8 Hz), 5.24 (d, 1H, J = 3.8 Hz), 3.84 (s, 3H), 3.69 (m, 4H), 3.47 (s, 2H), 2.43 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3, 148.3, 147.9, 143.7, 143.4, 139.8, 138.3, 135.2, 131.4, 130.5, 130.1, 128.3, 127.8, 127.7, 125.7, 120.5, 119.6, 101.5, 99.8, 67.2, 63.2, 62.0, 56.0, 53.9; ESI-MS *m/z* (M + H)$^+$ 474. Anal. ($C_{28}H_{28}ClN_3O_2$) C, H, N.

(±)-*N*-{(3-Chlorophenyl)[4-(morpholinomethyl)phenyl]methyl}-7-trifluoromethyl-4-aminoquinoline (4k). Starting from **7c** and **8c**, the title compound was prepared following the above-described procedure and was obtained as a white amorphous solid (48%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1H, J = 5.3 Hz), 8.27 (s, 1H), 7.94 (d, 1H, J = 8.8 Hz), 7.58 (d, 1H, J = 8.89 Hz), 7.36−7.26 (m, 8H), 6.32 (d, 1H, J = 5.3 Hz), 5.71 (d, 1H, J = 4.4 Hz), 5.62 (d, 1H, J = 4.4 Hz), 3.70−3.67 (m, 4H), 3.49 (s, 2H), 2.45−2.42 (m, 4H); ESI-MS *m/z* (M + H)$^+$ 512. Anal. ($C_{28}H_{25}ClF_3N_3O$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-6-chloro-2-methoxy-9-aminoacridine (4l). 7a (60 mg, 0.20 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine **12**[11] (100 mg, 0.30 mmol) were suspended in MeCN (5 mL), and glacial acetic acid (0.5 mL) was added. The resulting suspension was heated under reflux, and the progress of the reaction was monitored by TLC (20% MeOH in $CHCl_3$). After 3 h the solvent was removed under vacuum, and the residual gum was suspended in 10 mL of $CHCl_3$ and 10 mL of 1 N aqueous NaOH solution. The solid material was removed by filtration, the phases were separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 10 mL). The combined organic fractions were dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by column chromatography (20% MeOH in $CHCl_3$) to afford the title compound as a pale-yellow amorphous solid (32%): ^{1}H NMR (200 MHz, $CDCl_3$) 8.07 (d, 1H, J = 2.0 Hz), 7.97 (d, 1H, J = 9.7 Hz), 7.48 (d, 1H, J = 9.4 Hz), 7.35−7.29 (m, 7H), 7.20 (d, 2H, J = 7.9 Hz), 7.14 (dd, 1H, J = 2.0, 9.4 Hz), 6.84 (d, 1H, J = 2.6 Hz), 5.87 (d, 1H, J = 8.5 Hz), 4.80 (d, 1H, J = 8.8 Hz), 3.60 (s, 2H), 3.42 (s, 3H), 2.48 (m, 4H), 1.79 (m, 4H). ESI-MS *m/z* (M + H)$^+$ 542. Anal. ($C_{32}H_{29}Cl_2N_3O$) C, H, N.

(±)-*N*-{(4-Chlorophenyl)(phenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (5a). To a solution of **13a** (320 mg, 0.81 mmol) and a drop of *N*,*N*-dimethylformamide in dry CH_2Cl_2 (15 mL), a solution of $SOCl_2$ (180 μL, 2.54 mmol) in dry CH_2Cl_2 (10 mL) was added at 0 °C, and the mixture was stirred at 0 °C for 20 min and thereafter heated to 45 °C for 4 h. The volatiles were removed, and the residue was treated with dry CH_2Cl_2 (3 × 10 mL) and concentrated under reduced pressure in order to remove residual $SOCl_2$. The resulting hydrochloride salt was dissolved in THF (15 mL) and added to a refluxing mixture of sodium hydride (60% in mineral oil, 68.0 mg, 1.7 mmol) and 4-amino-7-chloroquinoline **15a** (0.31 mg, 1.7 mmol) in dry THF (20 mL). The resulting mixture was heated under reflux overnight and then was allowed to cool to 25 °C. Subsequently, the reaction mixture was quenched with ice and the aqueous phase was extracted with $CHCl_3$. The organic extracts were washed with brine, dried, and concentrated in vacuo. The residue was purified by flash chromathography (10% MeOH in $CHCl_3$) to afford **5a** as a pale-yellow oil (105 mg, 23%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, 1H, J = 5.2 Hz), 7.95 (d, 1H, J = 2.3 Hz), 7.74 (d, 1H, J = 8.8 Hz), 7.40−7.26 (m, 14H), 6.24 (bs, 1H), 5.86 (d, 1H, J = 5.7 Hz), 3.71 (s, 2H), 2.67−2.65 (m, 4H), 1.88−1.83 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.9, 149.1, 147.3, 143.8, 142.6, 141.9, 138.6, 134.8, 133.4, 130.4, 129.3, 129.1, 128.7, 128.6, 128.5, 128.4, 127.7, 125.8, 120.6, 117.7, 105.0, 71.2, 60.1, 54.3, 23.4; ESI-MS *m/z* (M + H)$^+$ 538. Anal. ($C_{33}H_{29}Cl_2N_3$) C, H, N.

***N*-{Bis(4-chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-7-chloro-4-aminoquinoline (5b).** Starting from **13b** (0.44 g, 1.46 mmol) and **15a**, the title compound was prepared following the above-described procedure to afford **5b** (12%) as a thick brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.97 (s, 1H), 7.73 (d, 1H, J = 8.7 Hz), 7.43 (d, 1H, J = 8.5 Hz), 7.28 −7.20 (m, 12H), 6.17 (s, 1H), 5.84 (s, 1H), 3.60 (s, 2H), 2.51 (m, 4H), 1.79 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.2, 149.3, 147.3, 142.7, 142.0, 139.3, 135.1, 133.9, 130.5, 129.6, 129.5, 128.9, 128.6, 126.2, 120.7, 117.8, 105.2, 71.0, 60.3, 54.6, 23.7; ESI-MS *m/z* (M + H)$^+$ 573. Anal. ($C_{33}H_{28}Cl_3N_3$) C, H, N.

***N*-{Bis(4-chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl}-6-chloro-2-methoxy-9-aminoacridine (5c).** Starting from **13b** (0.13 g, 0.31 mmol) and 6-chloro-2-methoxyacridin-9-amine **15b** (90 mg, 0.35 mmol), the title compound was prepared following the above-described procedure and was obtained as a yellow oil (38%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, 1H, J = 2.0 Hz), 7.83 (d, 1H, J = 9.3 Hz), 7.50 (d, 1H, J = 9.3 Hz), 7.35−7.32 (m, 4H), 7.23−7.17 (m, 5H), 7.12−7.05 (m, 4H), 7.98−6.92 (m, 1H), 6.75 (d, 1H, J = 2.3 Hz), 5.40 (s, 1H), 3.46 (s, 2H), 3.38 (s, 3H), 2.32 (m, 4H), 1.74 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 156.3, 148.0, 147.6, 147.1, 144.2, 142.6, 134.6, 133.7, 131.5, 130.6, 129.3, 128.9, 128.6, 128.1, 125.4, 125.3, 125.1, 121.9, 119.9, 105.0, 100.7, 74.6, 60.1, 55.3, 54.2, 23.6; ESI-MS *m/z* (M + H)$^+$ 654. Anal. ($C_{38}H_{32}Cl_3N_3O$) C, H, N.

Molecular Modeling. Molecular modeling calculations were performed on SGI Origin 200 8XR12000, while molecular modeling graphics were carried out on SGI Octane 2 and Octane workstations.

Apparent pK_a values of CQ, CLT, **3** and the newly designed compounds (**4a**−**l** and **5a**−**c**) in their tautomeric forms were estimated using the ACD/pK_a DB, version 11.00, software (Advanced Chemistry Development Inc., Toronto, Canada). Accordingly, percentages of neutral/ionized forms were computed at pH 7.4 (blood), pH 7.2 (cytoplasm), and pH 5.5 (*Pf* FV) using the Henderson−Hasselbach equation.

All the compounds were built taking into account the prevalent ionic forms of each tautomer at the considered different pH values using the Insight 2005 Builder module.

Partial charges of the compounds, considered protonated at the quinoline and acridine moieties as consequence of the estimation of apparent pK_a values, were assigned by comparing partial charges assigned by CFF91 force field[12] with those calculated by MNDO[13] semiempirical 1 SCF calculations performed on the neutral and the ionized compounds. In particular, CFF91 force field partial charges were added to the algebraic difference between MNDO partial charges of the protonated form and MNDO partial charge of the neutral form.

The conformational space of all compounds was sampled through 200 cycles of simulated annealing (CFF91 force field) by following our standard protocol. The system was heated to 1000 K over 2000 fs (time step of 3.0); a temperature of 1000 K was applied to the system for 2000 fs (time step of 3.0) with the aim of surmounting torsional barriers; successively temperature was linearly reduced to 300 K in 1000 fs (time step of 1.0). Resulting structures were subjected to energy minimization within the Insight 2005 Discover 3 module (CFF91 force field, conjugate gradient algorithm;[14] $\varepsilon = 80r$) until the maximum rms derivative was less than 0.001 kcal/Å and subsequently ranked in different families taking into account their (i) conformational energy, (ii) torsional angles values with the exception of those of lateral chains, and (iii) the presence/lack of hydrogen bonds.

In order to properly analyze the electronic properties, the most stable conformer of each family was subjected to a full geometry optimization by semiempirical calculations, using the quantum mechanical method AM1 in the Mopac 2007 package.[15] The GNORM value was set to 0.01. To reach a full geometry optimization, the criterion for terminating all optimizations was increased by a factor of 100, using the keyword PRECISE.

X-ray structures of iron complexes were selected and downloaded from the Cambridge Structural Database (CSD) using the CSDS (Cambridge Structural Database System) software Conquest 1.9. The resulting structures were superimposed on compounds **4a** and **4g** by fitting the iron interacting groups.

The dipole moments were calculated using partial charges obtained by the quantum mechanical method AM1 (Mopac 2007) and visualized as vector (Decipher, Accelrys, San Diego, CA).

Antimalarial Activity. (a) D10 and W2 Strains. *Pf* cultures were carried out according to Trager and Jensen's method with slight modifications.[18] The CQ-S strain D10 and the CQ-R strain W2 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 glutamine. 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 or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration of <1%, which is nontoxic to the parasite). Drugs were placed in 96-well, flat-bottom microplates (COSTAR), and serial dilutions were 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 (OD650) by measuring the activity of the pLDH according to a modified version of the method of Makler in control and drug-treated cultures.[16] 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.

(b) 3D7 and K1† Strains. All samples were tested in triplicate against the 3D7 and K1 strains. The cultures were maintained in continuous log phase growth in a RPMI1640 medium supplemented with 5% wash human A+ erythrocytes, 25 mM HEPES, 32 nM $NaHCO_3$, and Albu-MAXII (lipid-rich bovine serum albumin). All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Stock compound solutions were prepared in 100% DMSO at 5 mg/mL. The compounds were further diluted using a complete RPMI1640 medium supplemented with cold hypoxanthine and AlbuMAXII. Assays were preformed on sterile 96-well microtiter plates, with each plate containing 100 μL of the parasite culture (1% parasitemia, 2.5% hematocrit). After 24 h of incubation at 37 °C, 3.7 KBq of [^{3}H]hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filter mats, and the radioactivity was counted using a Wallac Microbeta 1450 scintillation counter.[17]

(c) NF54 and K1‡ Strains. *Pf* drug-sensitive NF54 and chloroquine-resistant K1 strains were cultivated in a variation of the medium previously described[18,19] consisting of RPMI 1640 supplemented with 0.5% ALBUMAX II, 25 mM Hepes, 25 mM $NaHCO_3$ (pH 7.3), 0.36 mM hypoxanthine, and 100 μg/mL neomycin. Human erythrocytes served as host cells. Cultures were maintained in an atmosphere of 3% O_2, 4% CO_2, and 93% N_2 in humidified modular chambers at 37 °C. Compounds were dissolved in DMSO (10 mg/mL), diluted in hypoxanthine-free culture medium, and titrated in duplicates over a 64-fold range in 96-well plates. Infected erythrocytes (1.25% final hematocrit and 0.3% final parasitemia) were added into the wells. After 48 h of incubation, 0.5 μCi of [^{3}H]hypoxanthine was added and plates were incubated for an additional 24 h. Parasites were harvested onto glass-fiber filters, and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations (IC_{50}) were estimated by linear interpolation.[20]

(d) In Vivo Antiplasmodial Activity Studies. In vivo tests were performed under the UK Home Office Animals (Scientific Procedures) Act 1986. Briefly, blood is taken from infected donor mice and diluted to a parasitemia of 1−2%. An inoculum of 0.2 mL (equivalent to 1×10^7 infected erythrocytes/mL) is administered to each mouse on day 0 by the intravenous route. At 2−4 h, postinoculation dosing commences. Experimental compounds were prepared in 10% Tween-80/EtOH and sterile PBS. Insoluble formulations were ball-milled and placed into a sonicating water bath, unheated, for 20−30 min. Compounds were administered in a 0.2 mL bolus every day for 4 days. The control drug, CQ, was administered po every day for 4 days at 10 mg/kg, giving >90% parasite clearance on day 4. On day 4 postinfection, blood smears of all animals (2 slides/mouse) were prepared, fixed with methanol, and stained with 10% Giemsa stain. Percentage parasitemia was determined microscopically. Results are reported as % infected erythrocytes and compared to the CQ control group and the untreated control group.

Pharmacokinetic Studies. Drug Administration and Plasma and Liver Sampling. Male CD1 albino mice, 25−30 g, and CD-COBS rats, 175−200 g (Charles River, Italy), were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L., n.116, G.U., Suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, 0J L358, 1, Dec 12, 1987; Guide for the Care and Use of Laboratory, U.S. National Research Council, 1996). A chronic jugular cannula (PE-50 tubing) was implanted in some rats under chloral hydrate anesthesia 24 h before the study started. These rats were given 5 mg/kg of the hydrochloride salt of compounds **4g** and **4a** intravenously in 0.9% NaCl, and serial blood samples were drawn at various times. Blood samples were assayed (30 and 180 min after dosing only, for the determination of **4g** blood-to-plasma ratio) and/or centrifuged to separate plasma, which was stored at −20 °C until the determination of **4a** and **4g** contents. Other rats were given **4g** orally (50 mg/kg, compound dissolved in ethanol/Tween-80, 30:70 v/v, and then diluted with water to a final concentration of 5 mg/mL). Mice too were given this compound orally (50 mg/kg, dissolved as above). The animals were sacrificed by decapitation under deep anesthesia 15, 30, 60, 120, and 180 min after dosing. Mixed arteriovenous trunk blood was collected in heparinized tubes and centrifuged at 3000*g* for 10 min, and the plasma was stored at −20 °C. Plasma was processed as described above. Liver was removed immediately, blotted with paper to remove excess surface blood, and quickly frozen in dry ice. Compounds **4g** and **4a** were extracted from plasma and liver homogenate by a liquid−liquid extraction procedure and quantified by reversed phase high-performance liquid chromatog-

raphy with fluorescence detection ($\lambda_{excitation}$ = 224 nm; $\lambda_{emission}$ = 420 nm). Briefly, to 0.05 mL of plasma or liver homogenate (1 g per 50 mL of 20 mM ammonium bicarbonate/MeOH, 50:50 v/v), 0.15 mL of 20 mM of ammonium bicarbonate, and 1 mL of hexane containing 1.5% of isoamilic alcohol were added. After agitation and centrifugation the surnatant was re-extracted with 0.15 mL of mobile phase, then recentrifuged. Then 0.1 mL of mobile phase was injected into the chromatographic column. Separation was done on a Brownlee Laboratory Spheri 5 RP 8 (4.6 mm × 250 mm, 5 μm). For **4g** the mobile phase consisted of 0.1 M MeCOONa/MeCN/MeOH/triethylamine (39.9/30/30/0.1, v/v) at pH 7. For **4a** the mobile phase consisted of 21.9% 0.1 M MeCOONa, 39% MeCN, and 39% MeOH with 0.1% triethylamine at pH 7. Both mobile phases were delivered isocratically at a flow rate of 1 mL/min. Standard curves were prepared by spiking blank mouse and rat plasma and liver homogenate. The within-day and day-to-day precision varied between −6% and 10%, regardless of the species and tissue analyzed. Over the sampling interval the plasma and brain area under the concentration−time curve (AUC_t) was determined using the linear trapezoidal rule. Then the value was extrapolated to infinity (AUC), when possible, using the terminal slope and the last plasma concentration. Other usual pharmacokinetic parameters were calculated by standard pharmacokinetic analysis. Plasma and liver concentrations were expressed as the mean ± SD.

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Supporting Information Available: Figure containing global minimum conformers of **4g** and **4a**; table of elemental analysis results for compounds **4a**−**l** and **5a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fidock, D. A.; Rosenthal, P. J.; Croft, S. L.; Brun, R.; Nwaka, S. Antimalarial drug discovery: efficacy models for compound screening. *Nat. Rev. Drug Discovery* **2004**, *3*, 509–520.

(2) (a) Towie, N. Malaria breakthrough raises spectre of drug resistance. *Nature* **2006**, *440*, 852–853. (b) White, N. J. Qinghaosu (artemisinin): the price of success. *Science* **2008**, *320*, 330–334.

(3) (a) Pagola, S.; Stephens, P. W.; Bohle, S. D.; Kosar, A. D.; Madsen, S. K. The structure of malaria pigment â-haematin. *Nature* **2000**, *404*, 307–310. (b) Ginsburg, H.; Famin, O.; Zhang, J.; Krugliak, M. Inhibition of glutathione-dependent degradation of heme by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. *Biochem. Pharmacol.* **1998**, *56*, 1305–1313.

(4) Müller, S. Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. *Mol. Microbiol.* **2004**, *53*, 1291–1305.

(5) (a) Gemma, S.; Kukreja, G.; Campiani, G.; Butini, S.; Bernetti, M.; Joshi, B. P.; Savini, L.; Basilico, N.; Taramelli, D.; Yardley, V.; Bertamino, A.; Novellino, E.; Persico, M.; Catalanotti, B.; Fattorusso, C. Development of piperazine-tethered heterodimers as potent antimalarials against chloroquine-resistant *P. falciparum* strains. Synthesis and molecular modelling. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3535–3539. (b) Gemma, S.; Kukreja, G.; Fattorusso, C.; Persico, M.; Romano, M. P.; Altarelli, M.; Savini, L.; Campiani, G.; Fattorusso, E.; Basilico, N.; Taramelli, D.; Yardley, V.; Butini, S. Synthesis of *N*1-arylidene-*N*2-quinolyl- and *N*2-acrydinylhydrazones as potent antimalarial agents active against CQ-resistant *P. falciparum* strains. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5384–5388. (c) Gemma, S.; Campiani, G.; Butini, S.; Kukreja, G.; Joshi, B. P.; Persico, M.; Catalanotti, B.; Novellino, E.; Fattorusso, E.; Nacci, V.; Savini, L.; Taramelli, D.; Basilico, N.; Morace, G.; Yardley, V.; Fattorusso, C. Design and synthesis of potent antimalarial agents based on clotrimazole scaffold: exploring an innovative pharmacophore. *J. Med. Chem.* **2007**, *50*, 595–598. (d) Gemma, S.; Campiani, G.; Butini, S.; Kukreja, G.; Sanna Coccone, S.; Joshi, B. P.; Persico, M.; Nacci, V.; Fiorini, I.; Novellino, E.; Fattorusso, E.; Tagliatatela-Scafati, O.; Savini, L.; Taramelli, D.; Basilico, N.; Parapini, S.; Morace, G.; Yardley, V.; Croft, S.; Coletta, M.; Marini, S.; Fattorusso, C. Clotrimazole scaffold as an innovative pharmacophore towards potent antimalarial agents. Design, synthesis, biological and structure−activity relationship studies. *J. Med. Chem.* **2008**, *51*, 1278–1294. (e) Fattorusso, C.; Campiani, G.; Kukreja, G.; Persico, M.; Butini, S.; Romano, M. P.; Altarelli, M.; Ros, S.; Brindisi, M.; Savini, L.; Novellino, E.; Nacci, V.; Fattorusso, E.; Parapini, S.; Basilico, N.; Taramelli, D.; Yardley, V.; Croft, S.; Borriello, M.; Gemma, S. Design, synthesis and structure−activity relationship studies of 4-quinolinyl- and 9-acrydinylhydrazones as potent antimalarial agents. *J. Med. Chem.* **2008**, *51*, 1333–1343.

(6) Gemma, S.; Kukreja, G.; Tripaldi, P.; Altarelli, M.; Bernetti, M.; Franceschini, S.; Savini, L.; Campiani, G.; Fattorusso, C.; Butini, S. Microwave-assisted synthesis of 4-quinolylhydrazines followed by nickel boride reduction: a convenient approach to 4-aminoquinolines and derivatives. *Tetrahedron Lett.* **2008**, *49*, 2074–2077.

(7) de Dios, A. C.; Tycko, R.; Ursos, L. M. B.; Roepe, P. D. NMR studies of chloroquine−ferriprotoporphyrin IX complex. *J. Phys. Chem. A* **2003**, *107*, 5821–5825.

(8) Angyal, S. J.; Angyal, C. L. Tautomerism of *N*-heteroaromatic amines. I. *J. Chem. Soc.* **1952**, 1461–1466.

(9) Peters, W.; Robinson, B. L. In *Handbook of Animal Models of Infection*; Zak., O., Sande, M., Eds.; Academic: London, 1999; pp 757−773.

(10) Parapini, S.; Basilico, N.; Pasini, E.; Egan, T. J.; Olliaro, P.; Taramelli, D.; Monti, D. Standardization of the physicochemical parameters to assess in vitro the β-hematin inhibitory activity of antimalarial drugs. *Exp. Parasitol.* **2000**, *96*, 249–256.

(11) Ferlin, M. G.; Marzano, C.; Chiarelotto, G.; Baccichetti, F.; Bordin, F. Synthesis and antiproliferative activity of some variously substituted acridines and azacridine derivatives. *Eur. J. Med. Chem.* **2000**, *35*, 827–837.

(12) Maple, J. R.; Hwang, M. J.; Stockfisch, T. P.; Dinur, U; Waldman, M.; Ewig, C. S.; Hagler, A. T. Derivation of class II force fields. I. Methodology and quantum force field for the alkyl functional group and alkane molecules. *J. Comput. Chem.* **1994**, *15*, 162–182.

(13) Dewar, M. J. S.; Thiel, W. Ground states of molecules. 38. The MNDO method. Approximations and parameters. *J. Am. Chem. Soc.* **1977**, *99*, 4899–4907.

(14) Fletcher, R. Unconstrained Optimization. In *Pratical Methods of Optimization*; John Wiley & Sons: New York, 1980; Vol. 1.

(15) Stewart, J. J. P. *MOPAC 2007*; Stewart Computational Chemistry: Colorado Springs, CO, 2007; http://OpenMOPAC.net.

(16) Makler, M.; Hinrichs, D. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205–210.

(17) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(18) Trager, W.; Jensen, J. B. Human malaria parasites in continuous culture. *Science* **1976**, *20*, 673–675.

(19) Dorn, A.; Stoffel, R.; Matile, H.; Bubendorf, A.; Ridley, R. G. Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein. *Nature* **1995**, *374*, 269–271.

(20) Huber, W.; Koella, J. A comparison of three methods of estimating EC_{50} in studies of drug resistance of malaria parasites. *Acta Trop.* **1993**, *55*, 257–261.

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