

Clotrimazole Scaffold as an Innovative Pharmacophore Towards Potent Antimalarial Agents: Design, Synthesis, and Biological and Structure–Activity Relationship Studies

Sandra Gemma,^{†,‡} Giuseppe Campiani,^{*,†,‡} Stefania Butini,^{†,‡} Gagan Kukreja,^{†,‡} Salvatore Sanna Coccone,^{†,‡} Bhupendra P. Joshi,^{†,‡} Marco Persico,^{†,§} Vito Nacci,^{†,‡} Isabella Fiorini,^{†,‡} Ettore Novellino,^{†,||} Ernesto Fattorusso,^{†,§} Orazio Tagliatela-Scafati,^{†,§} Luisa Savini,^{†,‡} Donatella Taramelli,^{†,⊥} Nicoletta Basilico,^{†,‡} Silvia Parapini,^{†,⊥} Giulia Morace,[⊥] Vanessa Yardley,^{†,▽} Simon Croft,^{†,▽} Massimiliano Coletta,[⊥] Stefano Marini,[#] and Caterina Fattorusso^{†,§}

European Research Centre for Drug Discovery and Development and Dipartimento Farmaco Chimico Tecnologico, Università di Siena, via Aldo Moro, 53100 Siena, Italy, Dipartimento di Chimica delle Sostanze Naturali and Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy, Dipartimento di Sanità Pubblica-Microbiologia-Virologia, Università di Milano, Via Pascal 36, 20133 Milano, Italy, Department of Infectious, Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom, and Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università degli Studi di Roma Tor Vergata, Rome, Italy

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We describe herein the design, synthesis, biological evaluation, and structure–activity relationship (SAR) studies of an innovative class of antimalarial agents based on a polyaromatic pharmacophore structurally related to clotrimazole and easy to synthesize by low-cost synthetic procedures. SAR studies delineated a number of structural features able to modulate the in vitro and in vivo antimalarial activity. A selected set of antimalarials was further biologically investigated and displayed low in vitro toxicity on a panel of human and murine cell lines. In vitro, the novel compounds proved to be selective for free heme, as demonstrated in the β -hematin inhibitory activity assay, and did not show inhibitory activity against 14- α -lanosterol demethylase (a fungal P450 cytochrome). Compounds **2**, **4e**, and **4n** exhibited in vivo activity against *P. chabaudi* after oral administration and thus represent promising antimalarial agents for further preclinical development.

Introduction

Despite the considerable efforts of academic and industrial research, malaria exacts a devastating social and economic cost across the globe. The disease causes 2–3 million deaths per annum as well as incalculable suffering. It strikes hardest at some of the poorest nations and is itself a significant cause and consequence of poverty. Its effects are not only limited to the developing nations, but malaria is of serious concern to travelers, especially in the absence of a vaccine and in the face of widespread resistance to several antimalarial drugs.¹

The etiological agent of malaria is *Plasmodium falciparum* (*Pf*), a highly evolved unicellular parasite whose life cycle, shared between an Anopheline mosquito vector and the human host, exhibits striking biological features that have been exploited to seek intervention strategies for malaria therapy. The detoxification of free heme released in the food vacuole (FV) of *Pf* during host hemoglobin (Hb) catabolism represents a unique biochemical process. It imposes a significant oxidative burden² that has to be managed by the plasmodium through specific mechanisms of heme detoxification such as crystallization of heme to hemozoin and degradation in the presence of reduced glutathione.³ Interference with such processes through

interaction with free heme and exacerbation of oxidative stress are supposed to be the mode of action of two of the most popular and effective classes of antimalarial agents, namely, 4-aminoquinolines and endoperoxides. Before the appearance of resistance, chloroquine (CQ) was one of the most successful antimicrobial agents ever developed, while artemisinins represent at the moment the most effective therapeutic option for the treatment of resistant malaria, although there is concern of overgrowing evidence for the in vitro appearance of artemisinin-resistant plasmodia.⁴ The high efficacy of these antimalarials resides in their ability to specifically target free heme (in its Fe(III) or Fe(II) forms, respectively) and to interfere with the fragile redox balance necessary for plasmodium survival. Moreover, their low propensity to induce resistance (resistance to CQ only occurred after many years of heavy drug pressure) is due to the lack of interaction with a specific protein target. Consequently, specific interaction with free heme aimed at inhibiting heme detoxification and increasing oxidative stress still represents a valuable strategy for the discovery of potential antimalarial drugs selectively toxic for the plasmodium.

Within a program aimed at developing new antimalarial drugs,⁵ we recently reported the identification of a new polyaromatic antimalarial pharmacophore based on clotrimazole (CLT, **1**, Chart 1) scaffold.⁶

CLT is a well-known antimycotic drug which exhibits a weak in vitro antimalarial activity against different *Pf* strains and importantly, irrespective of their CQ sensitivity.^{7,8} Biological activities of CLT are mediated by its ability to interact with ferri-protoporphyrin IX (Fe(III)-FP), which is present as the prosthetic group in several enzymes, such as (i) 14 α -lanosterol-demethylase (14-LD), the fungal cytochrome inhibited by CLT, (ii) human P450 cytochromes, which are inhibited by CLT causing altered metabolism of both xenobiotics and endogenous

* To whom correspondence should be addressed. Phone: 0039-0577-234172. Fax: 0039-0577-234333. E-mail: campiani@unisi.it.

[†] European Research Centre for Drug Discovery, Università di Siena.

[‡] Dipartimento Farmaco Chimico Tecnologico, Università di Siena.

[§] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli.

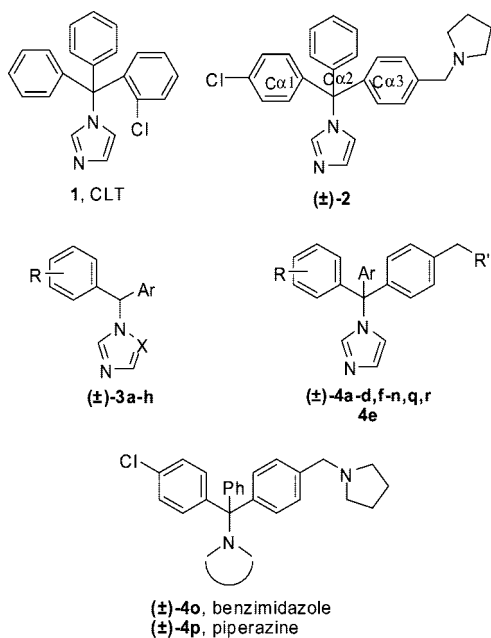
^{||} Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli.

[▽] London School of Hygiene and Tropical Medicine.

[⊥] Dipartimento di Sanità Pubblica-Microbiologia-Virologia, Università di Milano.

[#] Università degli Studi di Roma Tor Vergata.

Chart 1. Reference and Title Compounds



chemicals, and (iii) hemoperoxidase,⁹ a *Pf*-derived enzyme, which is inhibited by CLT, in the presence of H₂O₂, by a mechanism based on CLT-one electron oxidation product. Moreover, CLT is also able to form in vitro complexes with free Fe(III)-FP in which the imidazole ring behaves as a Fe(III) axial ligand and is able to inhibit in vitro the crystallization of free Fe(III)-FP into β -hematin.^{10,11}

Based on the aforementioned properties of CLT and considering its peculiar chemical structure, characterized by (i) an imidazole ring, known to mediate electron transfer reactions in biological systems,^{12,13} and (ii) a triphenylmethyl system, known to form and stabilize a radical intermediate (the triphenylmethyl radical was a landmark discovery as it marked the beginning of organic free radical chemistry¹⁴), we hypothesized that CLT, in the unique FV chemical environment, could interact with hemoglobin-derived ferro-protoporphyrin IX (Fe(II)-FP) and to get activated to form toxic trityl radicals able to kill the parasite by oxidative damage.

Consequently, our design strategy mainly focused on the synthesis of analogues with specific electronic and chemical features to better penetrate and accumulate into the FV and to generate active radical intermediates therein (pH 5.5 and presence of heme). Furthermore, we envisioned to improve the selectivity for free heme over heme as a prosthetic group to minimize side effects. These studies led to the identification of the CLT analogue **2**,⁶ a novel hit endowed with potent antimalarial activity in vitro whose structure is characterized by the presence of a protonatable pyrrolidinylmethyl group, which was rationally introduced to improve the pharmacokinetic properties, to increase tropism for the acidic FV and to attain selectivity for free heme over heme into cytochromes (due to the presence of conserved protonated amino acid residues at the lip of the cytochrome active site).

Here we report the synthesis and the biological evaluation of two series of compounds (**3a–h** and **4a–r**) based on the innovative polyaromatic pharmacophore previously disclosed.⁶ Structure–activity relationships for the novel series of antimalarials were investigated through structural modifications performed at the protonatable lateral chain of **2**, at the polyaromatic scaffold, introducing various heterocycles and substituted phenyl

rings, and at the heme complexing moiety. Among the compounds synthesized, the most active were selected for further biological investigation to establish (i) the antiparasmodial activity in vivo against murine plasmodia such as *P. berghei* and *P. chabaudi*, (ii) the antifungal activity predictive for their capability to interact with P450 cytochromes (protein-bound heme), and (iii) their ability to interact with Fe(III)-FP in vitro. Compounds **4e** and **4n** were identified as promising antimalarial agents suitable for further development.

Chemistry

Compounds **3a–g** reported in Table 1 were synthesized according to Schemes 1 and 2. Intermediate **7a** (Scheme 1) was synthesized starting from **6**,¹⁵ transformed into the corresponding Grignard reagent, and coupled with **5**, while **7b** was obtained starting from the bromo derivative **8**,⁶ after its transformation into the Boc-piperazine intermediate **9**. Subsequently, carbinols **7a,b** were transformed into the corresponding chlorides by exposure to thionyl chloride, followed by reaction with imidazole or 1*H*-1,2,4-triazole, in the presence of triethylamine, to afford the final compounds **3a–c**. Finally, treatment of *N*-Boc derivative **3c** with trifluoroacetic acid in dichloromethane (DCM) gave the free piperazine-derivative **3d**.

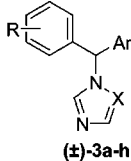
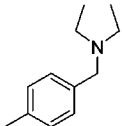
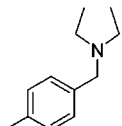
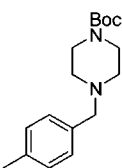
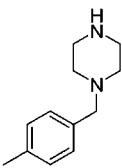
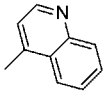
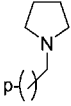
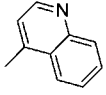
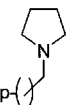
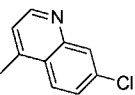
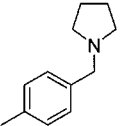
Compounds **3e–g** were synthesized as depicted in Scheme 2. Commercially available aldehyde **11a** was reacted with 3-chlorophenylmagnesium bromide to afford alcohol **13**, while carbinols **14a,b** were synthesized coupling the Grignard reagent obtained from **12** with the appropriate aldehydes **11a,b**. Aldehyde **11b** was in turn prepared starting from the 4,7-dichloroquinoline **10** by a two-step procedure involving the introduction of a methyl group at the 4-position¹⁶ of the quinoline system, followed by selenium dioxide oxidation. Conversion of carbinols **13** and **14a,b** into the corresponding chlorides, followed by treatment with the sodium salt of imidazole in *N,N*-dimethylformamide (DMF) afforded the final compounds **3e–g**.

Compounds **4a–e,o,p** were obtained as described in Scheme 3. The alkylation of bromobenzyl intermediate **15** with diethylamine, morpholine, or *N*-Boc-piperazine furnished the corresponding ketones **16a–c**. The latter were in turn reacted with phenylmagnesium bromide to afford carbinols **17a–c**. On the other hand, carbinols **18a–c** were prepared starting from **16d**,⁶ by reaction with *p*-fluorophenylmagnesium bromide (**18a**) or with the Grignard reagent derived from **12** (**18b**) or with phenylmagnesium bromide (**18c**). Following a described procedure,¹⁷ triarylcarbinols **17a–c** and **18a,b** were converted into the corresponding unstable chlorides which on reaction with imidazole in the presence of triethylamine afforded the target compounds **4a–e**. The chloride derivative of **18c**⁶ was used as a substrate for the synthesis of **4o,p** by reaction with benzimidazole or piperazine, respectively.

The heterocyclic analogues **4f–l** were prepared according to Scheme 4. 3-Chlorophenylmagnesium bromide was reacted with two equivalents of the corresponding commercially available carboxaldehydes **19a–e** to afford ketones **20a–e**, while the corresponding *p*-chloro-substituted derivatives **21a,b** were obtained starting from 4-chlorophenylmagnesium bromide and aldehydes **19a,b**. The ketones thus obtained were subjected to a second Grignard reaction to afford carbinols **22a–e** and **23a,b**, which were in turn transformed to final compounds **4f–l**, as described above.

For the synthesis of the quinoline-derivatives **4m,n** (Scheme 5), the intermediate ketones **26a,b** were prepared starting from 7-chloro-4-methoxyquinoline **24**.¹⁸ Accordingly, compound **24** was reacted with phenylacetonitrile or 4-fluorophenylacetonitrile, in the

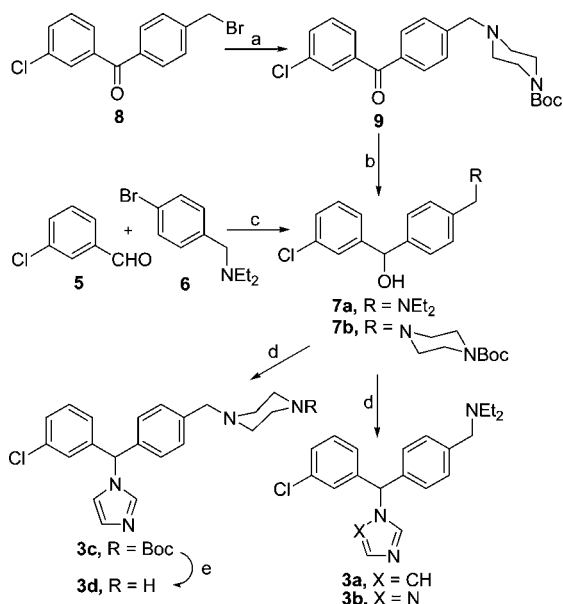
Table 1. Antiplasmodial Activity of Compounds 3a–h

 (±)-3a-h											
Compd	R	Ar	X	Ionic form (%) ^a			IC ₅₀ (nM) ^b				
				pH 7.4	pH 7.2	pH 5.5	D10 ^c	W2 ^d	3D7 ^c	K1 ^d	
3a	<i>m</i> -Cl		CH	DP(10) P(89) N(1)	DP(15) P(85) N(0)	DP(90) P(10) N(0)	1888	1293	452	282	
3b	<i>m</i> -Cl		N	P(99) N(1)	P(100) N(0)	P(100) N(0)	n.a.	n.a.	n.a.	3701	
3c	<i>m</i> -Cl		CH	DP(0) P(11) N(89)	DP(0) P(16) N(83)	DP(37) P(57) N(6)	5259	4109	1521	1071	
3d	<i>m</i> -Cl		CH	DP(10) P(88) N(2)	DP(15) P(84) N(1)	DP(90) P(10) N(0)	516	411	327	354	
3e	<i>m</i> -Cl		CH	DP(0) P(3) N(97)	DP(0) P(4) N(96)	DP(5) P(65) N(30)	n.a.	n.a.	n.a.	4287	
3f			CH	TP(0) DP(3) P(96) N(1)	TP(0) DP(5) P(95) N(0)	TP(5) DP(68) P(27) N(0)	7483	5034	1790	379	
3g			CH	DP(2) P(97) N(1)	DP(4) P(96) N(0)	DP(66) P(34) N(0)	1634	1808	1167	745	
3h ^e	<i>m</i> -Cl		CH	DP(10) P(89) N(1)	DP(15) P(85) N(0)	DP(90) P(10) N(0)	1419	1032	113	199	
CLT	-	-	-	P(5) N(95)	P(8) N(92)	P(81) N(19)	550	490	60	250	
CQ				DP(8) P(92) N(0)	DP(12) P(88) N(0)	DP(87) P(12) N(0)	22	280	10	260	

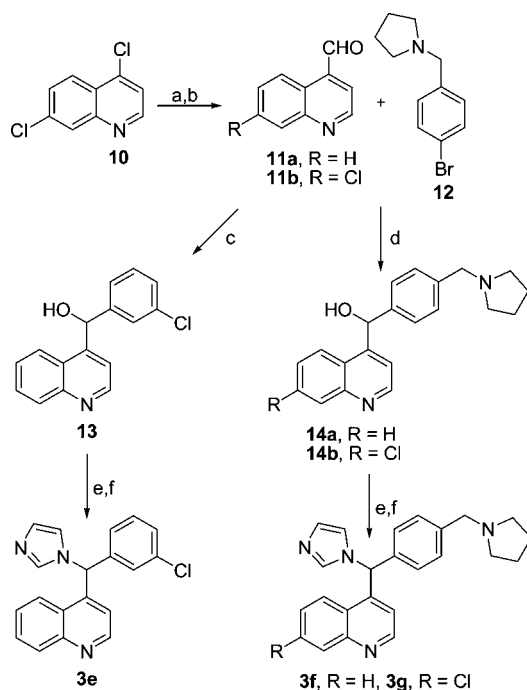
^a Percentage of ionic form in brackets; TP = triprotonated form; DP = diprotonated form; P = protonated form; and N = neutral form (ACD/pKa DB version 10.00 software – Advanced Chemistry Development, Inc., Toronto, Canada-). ^b IC₅₀s are the mean of at least three determinations. Standard errors were all within 10% of the mean; n.a. = Not Active (IC₅₀ > 10 μM). ^c CQ-S clone. ^d CQ-R clone. ^e Ref. 6.

presence of sodium hydride, to afford nitriles **25a** and **25b**, respectively, which were subsequently converted into the corre-

sponding ketone derivatives **26a,b**.¹⁹ Following the synthetic procedure already described, Grignard reaction, followed by

Scheme 1^a

^a Reagents and conditions: (a) *N*-Boc-piperazine, Et₃N, MeCN, 0 °C, 2 h; (b) NaBH₄, THF/H₂O 2:1, 80 °C, 1.5 h; (c) Mg turnings, THF, 80 °C; (d) (i) SOCl₂, DCM, 0 °C to rt, 4 h; (ii) imidazole (for **3a,c**) or 1*H*-1,2,4-triazole (for **3b**), Et₃N, MeCN, 0 to 80 °C, 4 h; (e) TFA, DCM, 0–5 °C, 3 h.

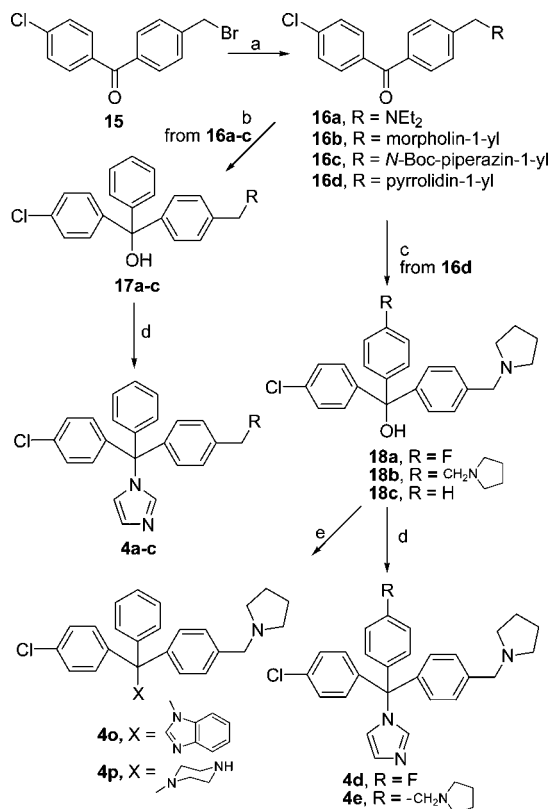
Scheme 2^a

^a Reagents and conditions: (a) MeMgBr, [1,2-bis(diphenylphosphino)ethane]dichloronickel(II), Et₂O, 0 °C to rt, 24 h; (b) SeO₂, bromobenzene, 170–175 °C, 18 h; (c) 3-chlorophenylmagnesium bromide, THF, 80 °C, 6 h; (d) *n*-BuLi, THF, –78 °C to rt, 24 h; (e) SOCl₂, DCM, 0 °C to rt, 5 h; (f) imidazole sodium salt, DMF, 80 °C, 3 h.

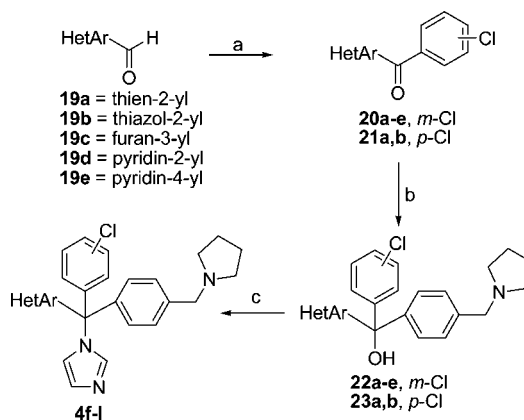
chlorination of the resulting carbinols **27a,b** and subsequent treatment with imidazole and triethylamine, afforded the desired compounds **4m,n**.

Results and Discussion

1. In Vitro Antimalarial Activity and Structure–Activity Relationship. All synthesized compounds were tested in vitro against a series of *Pf* strains, namely, the CQ-sensitive

Scheme 3^a

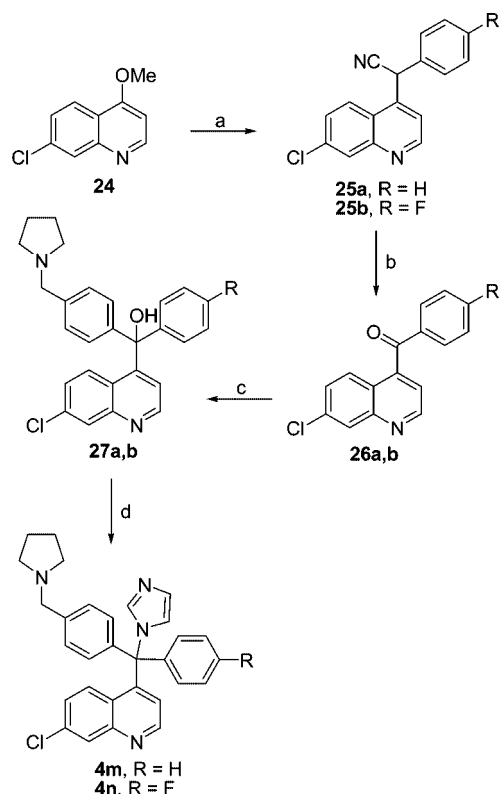
^a Reagents and conditions: (a) Et₃NH (for **16a**), morpholine (for **16b**), *N*-Boc-piperazine (for **16c**), Et₃N, MeCN, 0 °C, 1 h; (b) phenylmagnesium bromide, THF, 80 °C, 6 h; (c) *p*-fluorobromobenzene (for **18a**), **12** (for **18b**), Mg, THF, 80 °C, 6 h; (d) (i) SOCl₂, DCM, 0 to 45 °C, 4 h, (ii) imidazole, Et₃N, MeCN, 0 to 80 °C, 4 h; (e) (i) SOCl₂, DCM, 0 °C to rt, 4 h, (ii) benzimidazole (for **4o**), piperazine (for **4p**), Et₃N, MeCN, 0 to 80 °C, 4 h.

Scheme 4^a

^a Reagents and conditions: (a) *m*-chlorophenylmagnesium bromide or *p*-chlorophenylmagnesium bromide, THF, 80 °C, 3 h; (b) **12**, Mg, THF, 80 °C, 6 h; (c) (i) SOCl₂, DCM, 0 °C to rt, 4 h; (ii) imidazole, Et₃N, MeCN, 0 to 80 °C, 4 h.

(CQ-S) D10 and 3D7 and the CQ-resistant (CQ-R) W2 and K1 strains. The antimalarial activity (IC₅₀, nM) was quantified as inhibition of parasite growth, measured with the production of parasite lactate dehydrogenase (D10 and W2 strains, asynchronous culture) or the incorporation of [³H]-hypoxanthine (3D7 and K1 strains, synchronous culture). Results are reported in Tables 1,2.

Based on the above-mentioned CLT properties, we used its scaffold to develop novel antimalarial agents characterized by

Scheme 5^a

^a Reagents and conditions: (a) phenylacetonitrile (for **25a**), 4-fluorophenylacetonitrile (for **25b**), NaH (60% in mineral oil), THF, 70 °C, 30 min; (b) NaH (60% in mineral oil), O₂, THF, rt, 2 h; (c) **12**, Mg, THF, 80 °C, 6 h; (d) (i) SOCl₂, DCM, 0 to 45 °C, 24 h, (ii) imidazole, Et₃N, MeCN, 0 to 80 °C, 4 h.

the selective binding to free heme but lacking interaction with a definite *Pf* or host target protein (to minimize resistance), with improved antimalarial potency and pharmacological profile over CLT and reduced side effects. To achieve activity against both CQ-S and CQ-R *Pf* strains, we reasoned that an effective antimalarial should rapidly accumulate and be activated inside the microenvironment of the parasite FV (i.e., pH 5.5; redox milieu –250 mV; presence of free heme), where the spontaneous oxidation of hemoglobin-derived Fe(II) heme to Fe(III) heme promotes the formation of superoxide ions generating H₂O₂ and hydroxyl radicals. On these bases, our design strategy focused on novel antimalarials characterized by improved penetration into the FV and with appropriate electronic features that would generate radical intermediates in the *Pf*FV environment. Starting from CLT structure, to increase FV accumulation, we introduced an extra protonatable function, while to modulate the interaction with free heme and the generation and stabilization of a radical intermediate, we investigated the effect of different heme complexing groups and of different aromatic substituents.

(a). **Diarylmethyl versus Triarylmethyl Derivatives.** We dismantled the structure of CLT and consequently synthesized the diphenylmethyl derivatives **3a–g** (Table 1) to identify the minimal structural requirement for antimalarial activity, which could subsequently be optimized to meet the pharmacodynamic and pharmacokinetic prerequisites to enter into further development. Compounds **3a–g** were found to be significantly less potent than CLT against both CQ-S and CQ-R *Pf* strains (Table 1). According to our hypothesized mechanism of action, the lower activity of the diaryl analogues with respect to CLT (triaryl system) can be explained in terms of interference with the formation and stabilization of a radical intermediate, critical

for antimalarial activity. Indeed, the potency strongly increased when an additional phenyl ring or an appropriate heterocycle was introduced (**3h** vs **4q** and **4g**, **3g** vs **4m**, Tables 1 and 2), indicating that a triarylmethyl moiety represents the minimal feature for high antimalarial activity.

(b). **Key Role of the Heme Complexing Group.** Following our design hypothesis, the imidazole ring, protonated at the FV pH and having the dual role of interacting with free heme and of being the substrate for the redox reaction generating a trityl radical, was the heme interacting group of choice.^{9,12,13,20} In the diarylmethyl series, introduction of a triazole ring resulted in the loss of antimalarial activity with respect to the imidazole analogue (**3a** vs **3b**), and in the triarylmethyl series, a similar effect was observed when a piperazine ring was introduced (**4p** vs **2**). This confirms that the introduction of groups capable of chelating iron but unable to promote the formation of the radical intermediate are detrimental for the activity. Indeed, the triazole nitrogen-centered radical is destabilized through the unfavorable dipole–dipole interaction of the radical electron with lone pair electrons of the adjacent nitrogen atom.²⁰ Moreover, the triazole moiety is not protonated at the FV pH, causing a decreased accumulation of the compound. On the other hand, the piperazine nitrogen is protonated at the FV pH but is unable to generate a radical through a conjugation-mediated electron transfer. Accordingly, **4o** was found to be more potent than **4p** but slightly less potent than **2** because its benzimidazole ring is able to chelate iron and to generate free radicals²¹ but is less protonated at FV pH than imidazole. This finding further supports our design hypothesis.⁶

(c). **Protonatable Lateral Chain.** Introduction of an extra protonatable chain was a key finding to highly improve potency and FV accumulation of the novel antimalarials (**2** vs CLT).⁶ The antimalarial activity could also be modulated by varying the nature of the protonatable lateral chain. Substitution of the pyrrolidine group by a piperazine led to a moderate increase of antimalarial activity against asynchronous D10 and W2 strains (**3d** vs **3h** and **4c** vs **2**). This data could be explained taking into consideration that also piperazine is able to form complexes with iron,^{5a} thus suggesting that an additional metal interaction point, as in **3d** and **4c**, could be responsible of the increased in vitro antimalarial activity with respect to **2** (a mechanism already exploited by other antimalarial agents used for iron chelation therapy²²). In the triarylmethyl series, the introduction of a diethylamino group (**4a**) resulted in a decreased activity against both CQ-S strains, while a 10-fold potentiation was observed against the K1 CQ-R strain (**4a** vs **2**). On the other hand, the presence of the less protonatable morpholine ring (**4b**) reduced the antimalarial activity with respect to **2** against both D10 and W2 strains. Such a dependence of antimalarial activity from the nature of the protonatable lateral chain has been extensively described for CQ derivatives, in which introduction of bulkier groups at the distal nitrogen generally resulted in an increased activity against CQ-R strains²³ and is probably suggestive of a possible altered interaction with a protein transporter, which has been hypothesized as a mechanism leading to CQ-resistance.

(d). **Halogen vs Methoxy Substituents at the Trityl System.** Electron-donating and electron-withdrawing substituents at the trityl system differently affect the capability of the system to generate and stabilize radical intermediates and, by consequence, the antimalarial potency of the corresponding compounds. In general, electron-donating or -withdrawing groups may significantly modulate the reactivity of triarylmethyls and, consequently, the pharmacological properties. In fact, while the *p*-Cl substituent was responsible for a fine-tuning

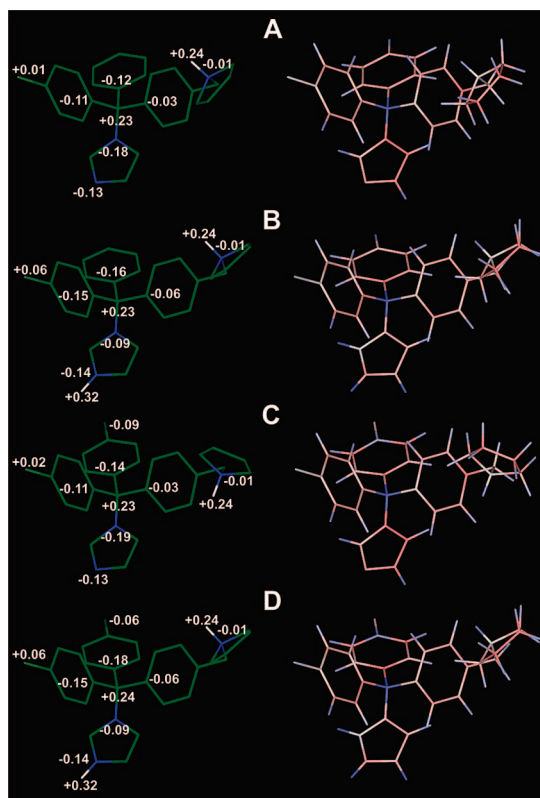
Table 2. Antiplasmodial Activity of Compounds 4a–r

Compd	R	Ar	R'	Ionic form (%) ^a			IC ₅₀ (nM) ^b			
				pH 7.4	pH 7.2	pH 5.5	D10 ^c	W2 ^d	3D7 ^c	K1 ^d
				DP(7) P(93) N(1)	DP(10) P(90) N(0)	DP(85) P(15) N(0)				
4a	p-Cl			DP(7) P(93) N(1)	DP(10) P(90) N(0)	DP(85) P(15) N(0)	415	n.t.	41.4	0.48
4b	p-Cl			DP(2) P(28) N(71)	DP(3) P(37) N(59)	DP(82) P(18) N(1)	726	561	n.t.	n.t.
4c	p-Cl			DP(7) P(91) N(2)	DP(11) P(88) N(1)	DP(86) P(14) N(0)	84.1	55.4	n.t.	n.t.
4d	p-Cl			DP(6) P(93) N(1)	DP(9) P(90) N(0)	DP(83) P(17) N(0)	118	82.0	1.5	380
4e	p-Cl			TR(6) DP(92) P(2) N(0)	TR(10) DP(89) P(1) N(0)	TR(84) DP(16) P(0) N(0)	48.4	26.1	39.2	11.7
4f	m-Cl			DP(5) P(94) N(1)	DP(8) P(92) N(0)	DP(80) P(20) N(0)	7170	4267	1044	1268
4g	m-Cl			DP(1) P(98) N(1)	DP(1) P(98) N(1)	DP(42) P(58) N(0)	484	314	1850	66.7
4h	m-Cl			DP(8) P(91) N(1)	DP(12) P(88) N(0)	DP(87) P(13) N(0)	238	101	167	n.t.
4i	m-Cl			DP(2) P(97) N(1)	DP(3) P(97) N(1)	DP(60) P(40) N(0)	619	349	157	155
4j	m-Cl			TR(0) DP(3) P(96) N(1)	TR(0) DP(5) P(94) N(1)	TR(16) DP(60) P(23) N(0)	1228	587	415	510
4k	p-Cl			DP(5) P(94) N(1)	DP(8) P(91) N(0)	DP(82) P(18) N(0)	1357	3571	6689	1778
4l	p-Cl			DP(1) P(98) N(1)	DP(1) P(98) N(2)	DP(44) P(56) N(0)	300	177	80	180
4m	H			DP(2) P(97) N(1)	DP(3) P(96) N(1)	DP(61) P(39) N(0)	168	148	10	60
4n	p-F			TP(0) DP(2) P(97) N(1)	TP(2) DP(2) P(97) N(1)	TP(0) DP(58) P(42) N(0)	85.8	66.9	2.0	67
4o	-	-	-	DP(0) P(99) N(1)	DP(1) P(99) N(0)	DP(25) P(75) N(0)	1029	379	376	23.0
4p	-	-	-	DP(97) P(3) N(0)	DP(97) P(3) N(0)	DP(100) P(0) N(0)	n.a.	3845	1560	3510
4q ^e	m-Cl			DP(6) P(93) N(1)	DP(10) P(90) N(0)	DP(84) P(16) N(0)	217	102	46.7	23.3
4r ^e	p-Ome			DP(9) P(90) N(1)	DP(13) P(87) N(0)	DP(88) P(12) N(0)	2040	1840	8130	5933
2 ^e	p-Cl			DP(7) P(93) N(1)	DP(10) P(89) N(0)	DP(85) P(15) N(0)	130	59	7.0	5.0
CLT	-	-	-	P(5) N(95)	P(8) N(92)	P(81) N(19)	550	490	60	250
CQ	-	-	-	DP(8) P(92) N(0)	DP(12) P(88) N(0)	DP(87) P(12) N(0)	22	280	10	260

^a Percentage of ionic form in brackets; TP = triprotonated form; DP = diprotonated form; P = protonated form; and N = neutral form (ACD/pKa DB version 10.00 software – Advanced Chemistry Development, Inc., Toronto, Canada-). ^b IC₅₀s are the mean of at least three determinations. Standard errors were all within 10% of the mean; n.t. = Not Tested. ^c CQ-S clone. ^d CQ-R clone. ^e Ref. 6.

Table 3. AM1 Partial Charges of the C α (*ipso* Phenyl Carbons) and the Central Methine Carbon of **2**, **4d**–**4h**, **4q**, and **4r** in their Prevalent Ionic Forms at Cytoplasmatic (7.2) and FV (5.5) pH

cmpd	C α 1		C α 2		C α 3		central methine carbon	
	P ^a	DP ^a	P	DP	P	DP	P	DP
2	-0.11	-0.15	-0.12	-0.16	-0.03	-0.06	+0.23	+0.23
4d	-0.11	-0.15	-0.14	-0.18	-0.03	-0.06	+0.23	+0.24
4e^b	-0.12	-0.18	-0.07	-0.09	-0.05	-0.08	+0.22	+0.24
4f	-0.11	-0.14	-0.45	-0.54	-0.04	-0.07	+0.27	+0.28
4g	-0.10	-0.15	-0.36	-0.45	-0.04	-0.07	+0.26	+0.28
4h	-0.10	-0.14	-0.19	-0.26	-0.05	-0.07	+0.26	+0.28
4n	-0.14	-0.18	-0.06	-0.11	-0.04	-0.06	+0.23	+0.24
4q	-0.10	-0.13	-0.11	-0.16	-0.04	-0.07	+0.23	+0.24
4r	-0.15	-0.20	-0.11	-0.15	-0.04	-0.06	+0.24	+0.24

^a P = protonated form; pH 7.2; DP = diprotonated form; pH 5.5.^b Compound **4e** can only exist in the diprotonated (pH 7.2) and triprotonated (pH 5.5) form.**Figure 1.** Resulting MOPAC (AM1) conformers of **2** and **4d** in their prevalent ionic forms at the cytoplasmatic pH (A and C, respectively) and at the FV pH (B and D, respectively). Left: the compounds are colored by atom type (green for C, white for H, blue for N, light-green for Cl, and dark-green for F). AM1 partial charges of C α (*ipso* phenyl carbons), central methine carbon, heteroatoms, and protonatable groups are shown. All hydrogens, except those of protonable nitrogens, have been omitted for clarity. Right: the compounds are colored by AM1 charge distribution (the atoms are colored from red (−0.5) to blue (+0.5) depending upon their partial charge value).

of activity against CQ-S and CQ-R strains (**2** vs **4q**), a dramatic decrease of activity was observed when the electron-withdrawing chlorine atom was replaced by the electron-donating methoxy group (**4r** vs **2**). AM1 quantomechanical calculation showed that the partial charge distribution at the trityl system in the monoprotinated and diprotonated forms of both **2** and **4r** was highly affected by the *p*-substituent (Table 3). In **4r**, the presence of the *p*-methoxy-substituted phenyl ring determined an increased electron density at the C α 1 (*ipso* phenyl carbon 1) linked

to the trityl central methine carbon (Chart 1), which is detrimental for the formation of the intermediate trityl radical.

We also explored the effect produced by the introduction of fluorine atoms at the trityl system to increase the metabolic stability. When a *p*-fluorine substituent was introduced, the resulting compound **4d** showed antimalarial properties similar to **2** against asynchronous strains D10 and W2 and the synchronous CQ-S strain 3D7. However, it proved to be less-potent against K1. This could be explained considering that each *Pf* strain, sensitive or not to CQ, may present a different chemical environment at the FV level (i.e., pH value associated to CQ resistance²⁴). Thus, in the case of **4d**, the efficacy on the different strains can be explained by taking into consideration the electron distribution of the monoprotinated and diprotonated forms whose relative amount is determined by the different FV environment of each strain (Table 2). Accordingly, the diprotonated form of **4d** has a partial charge distribution disfavoring radical formation with respect to the corresponding monoprotinated form (Figure 1 and Table 3).

(e). Modulation of the Electronic Features of the Triaryl-methyl System: Effect of Five-Membered Heterocycles. To further explore the role played by specific electronic features in modulating the formation/stabilization of a radical intermediate and the antimalarial activity of these polycyclic antimalarials, we replaced one aromatic group by five-membered heterocycles and their partial charge distribution was calculated by AM1 quantomechanical methods. Even though it has been demonstrated that replacement of a phenyl by a thiophene does not change dramatically the reactivity of the trityl system, we observed in our series of compounds (five-membered heterocycles combined to the presence of an imidazole ring) significantly different electron distributions in the polyaromatic systems taken into consideration. Indeed, as shown in Figure 2 and Table 3, by comparison of the electron distribution of **4q**, to the one of the thiophene (**4f**), thiazole (**4g**), and furan (**4h**) derivatives, an increased electron density at the C α (*ipso* aryl carbons, Chart 1) was observed, thus reducing the generation of a radical intermediate and lowering the antimalarial potency with respect to **4q**. In general, the increased polarization and electron density of the heterocycles introduced was inversely related to their antimalarial potency (Figure 2). In fact, the thiophene derivative **4f**, which displayed the higher electron density at C α was the least potent compound of the subseries, while activity increased introducing a furan or a thiazole ring, following the rank order of potency **4h** > **4g** > **4f**. The same SAR was observed for the corresponding *p*-Cl analogues **4k** and **4l**. The higher activity of **4l** compared to **4g** confirmed the fine-tuning of the antimalarial activity operated by the *p*-Cl substituent even in this heterocyclic series of analogues.

(f). Pyridine versus Quinoline: Increasing the Antimalarial Potency. When a 4-pyridyl (**4j**) or a 2-pyridyl (**4i**) heterocycles were taken into consideration, a lower antimalarial activity with respect to **4q** was observed. Although the replacement of a phenyl ring by a pyridyl ring in a triphenylmethyl radical does not change dramatically its stability, however, the pyridyl group delocalizes radicals less than the phenyl ring.¹⁴ On these bases, to increase the ability to stabilize a radical intermediate and, consequently, the antimalarial activity, we planned to replace the 4-pyridyl by a 7-chloroquinolin-4-yl system (a pyridine with a fused 4-Cl benzo group at C2–C3), which stabilizes the radical by a higher π delocalization. Accordingly, the 7-Cl-quinoline derivative **4m**, although lacking the favorable electron-withdrawing substituent on the phenyl ring (see paragraph (d), Halogen vs Methoxy Substituents at

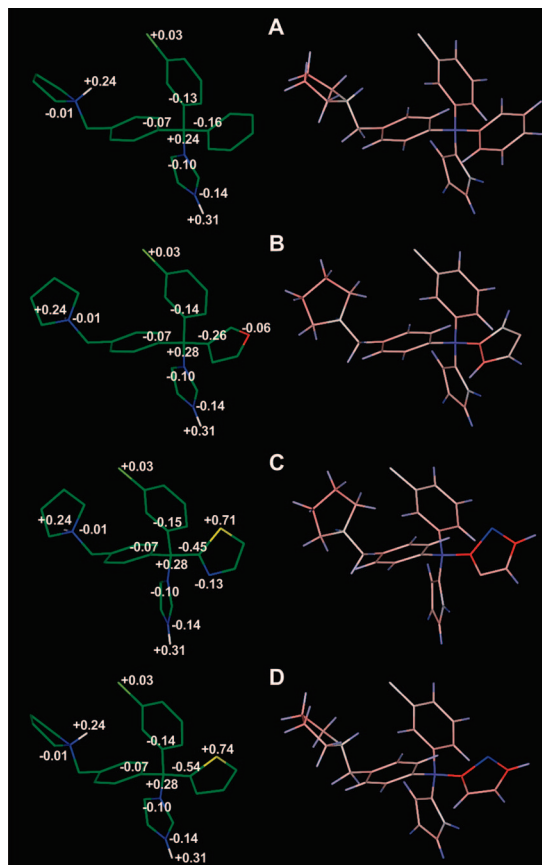


Figure 2. Resulting MOPAC (AM1) conformers of **4q** (A), **4h** (B), **4g** (C), and **4f** (D) in their prevalent ionic forms at the FV pH. Left: the compounds are colored by atom type (green for C, white for H, blue for N, light-green for Cl, red for O, and yellow for S). AM1 partial charges of C α (*ipso* phenyl carbons), central methine carbon, heteroatoms, and protonatable groups are showed. All hydrogens, except those of the protonable nitrogens, have been omitted for clarity. Right: the compounds are colored by AM1 charge distribution (the atoms are colored from red (−0.5) to blue (+0.5) depending upon their partial charge value).

the Trityl System), was endowed with a higher antimalarial activity against both CQ-R and CQ-S strains than **4j**, in perfect agreement with its electronic properties. The higher potency of **4m** might also be explained taking into account that the 7-chloroquinoline system may interact with heme, competing with the imidazole. According to the SARs discussed in paragraph (d), a further increase of activity was then obtained by introducing a *p*-F substituent (**4n**) at the phenyl ring of **4m** (**4n** vs **4m**). Nevertheless, contrary to what was observed for compound **4d**, the activity on the K1 strain of **4n** is preserved (Table 2), according to its partial charge distribution (Table 3).

(g). Achiral Antimalarials. Most of the compounds designed and tested incorporate a chiral center that, when resolved into the pure enantiomers, may cause an increase of synthetic costs, thus compromising the potentiality of industrial development. Consequently, to improve the drugability of these antimalarials, we specifically removed the chiral center by introducing an extra protonatable pyrrolidinylmethyl system (**4e**), maintaining significant FV penetration and accumulation and ameliorating the solubility (**2** logD: pH 7.4 = 3.33, pH 7.2 = 3.13, pH 5.5 (FV) = 1.72; **4e** logD: pH 7.4 = 1.56, pH 7.2 = 1.40, pH 5.5 (FV) = 0.67). Similar to **2**, compound **4e** was found to be highly potent against all tested strains and was more potent against CQ-R than CQ-S strains. In addition, the presence of two pyrrolidinylmethyl substituents decreased the electron density

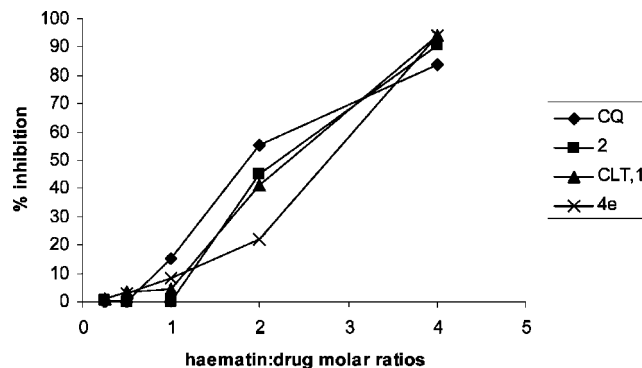


Figure 3. Hematin inhibitory activity assay of compounds CQ, CLT, **2**, and **4e**.

Table 4. β -Hematin Inhibitory Activity Assay of Compounds **4e**, CLT (**1**), and CQ

cmpd	IC ₅₀ ^a
4e	2.85
2	2.53
CLT, 1	2.50
CQ	1.69

^a The IC₅₀ 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.

at C α (Chart 1; AM1 calculations, Table 3), increasing the stabilization of a putative trityl radical intermediate responsible for antimalarial activity. These data together with the high potency of **4e** further confirm the hypothesis of the generation of a radical intermediate for this class of antimalarials. Compound **4e** was thus selected for further biological investigation (see paragraph 5).

2. β -Hematin Inhibitory Activity Assay. Two of the most active compounds (**2** and **4e**) were screened for inhibition of β -hematin formation by using the β -hematin inhibitory activity (BHIA) assay.²⁵ Both compounds showed a dose-dependent inhibition of β -hematin formation (Figure 3 and Table 4). Although compounds **2** and **4e** were found much more potent than CLT on different *Pf* strains, their in vitro BHIA was found to be very similar to that of CLT, suggesting a similar interaction with free heme. On the other hand, although with a similar protonation state, CQ and **2** (Table 2) differ for their in vitro antimalarial potency (**2** >> CQ) and their inhibitory potency in the BHIA assay (CQ >> **2**). This suggests that other physicochemical characteristics of the molecules and/or a different mechanism of action, besides inhibition of hemozoin formation, may be responsible for killing the parasite. Compound **4e**, triprotonated at the FV pH (Table 2), proved to be even less potent than CQ and **2** in the BHIA assay, while maintaining a high potency against CQ-S and CQ-R strains.

3. Antifungal Activity. Selected compounds were also tested to measure their antifungal activity (Table 1, Supporting Information (SI)), as the lack of antifungal activity could be predictive for a low propensity to interfere with cytochrome P450 activity. CLT and the other antifungal azoles are known to exert their activity by interfering with fungal cytochrome P450s (such as 14-LD), and some of their side effects are linked to interference with human cytochromes. On the basis of our docking studies and bioinformatic analysis, the basic side chain of **2** and analogues, besides to increase the pharmacokinetic properties with respect to CLT, were also designed to improve selectivity for free heme over heme as cytochromes P450 prosthetic group. To hit this mark, CLT was subjected to flexible

Table 5. Cytotoxicity Assays for Selected Compounds

cmpd	TC ₅₀ (μM) ^a			
	NSO cells ^b	Daudi cells ^c	Normal human lymphocytes ^d	KB cells ^e
2	51.5	58.5	70.3	n.t. ^f
4b	16.0	15.6	13.3	n.t.
4c	16.2	15.8	13.7	n.t.
4d	n.t.	n.t.	n.t.	8.2
4e	47.0	58.7	68.5	n.t.
4g	52.9	57.5	80.5	n.t.
4i	n.t.	n.t.	n.t.	27.2
4l	n.t.	n.t.	n.t.	64.2
4m	n.t.	n.t.	n.t.	51.8
4n	n.t.	n.t.	n.t.	33.0
4p	n.t.	n.t.	n.t.	20.9
CLT	n.t.	n.t.	n.t.	88.1
CQ	n.t.	n.t.	n.t.	207.0

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

Table 6. In Vivo Antimalarial Activity of Selected Compounds against *P. berghei* and *P. chabaudi* after Oral Administration

cmpd	% suppression on day 4 ^a (mg/Kg)	
	<i>P. chabaudi</i> AS	<i>P. berghei</i> ANKA
2	94.8 (50) ^b	37.6 (30) ^c
4e	94.0 (50) ^b	n.t. ^d
4g	73.1 (50) ^b	n.t.
4n	99.4 (50) ^b	n.t.
CLT (1)	6.0 (50) ^b	28.3 (150) ^b
CQ	100 (10) ^b	89.1 (10) ^b

^a Percent suppression = [(C - T)/C] × 100; where C = parasitaemia in control group and T = parasitaemia in treated group after oral/i.p. administration. Five mice per group were used. ^b Oral administration. ^c Intraperitoneal administration. ^d n.t. = not tested.

docking studies into 14-LD homology model²⁶ of *C. albicans*. At this purpose, it has to be underlined that the unfavorable electronic and steric interactions with the residues present in the active site of 14-LD may not be the only determinant for substrate selectivity, as the external surface and the access channel properties may play a key role.²⁷ Indeed we considered the presence of positively charged residues on the external surface of the active site and in the putative substrate access channel of 14-LD. Most of the positively charged residues identified in *C. albicans* 14-LD, including the K108 at the active site, are conserved in the other fungi P450 orthologs as well as in the active site of CYP3A4, the most important enzyme for drug metabolism in humans, where two arginine residues are placed in the same region occupied by K108. Our studies led to the hypothesis that the introduction of a protonatable group could decrease interaction with heme as P450s prosthetic group.

These results were confirmed by determination of the antifungal activity of compounds **2**, **4e**, and **4n** against a panel of fungal species (*Aspergillus* spp., *Candida* spp., and one isolate of *Cryptococcus neoformans*). As shown in the SI, Table 1, none of the new compounds had any significant activity against these fungi, confirming the selectivity for free heme as the key factor for antimalarial activity of these innovative antimalarial agents.

4. Cytotoxicity: MTT Assays. Cytotoxicity for compounds **2** and **4b,c,e,g** was evaluated using plasmocytoma murine cell line (NSO cells), normal human lymphocytes PHA-stimulated, and human lymphoblastoid cell line (Daudi cells). Data are shown in Table 5 and were obtained by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test aimed to analyze cell proliferation in cells cultured in the presence of

different compounds.²⁸ The data processing included the Student's *t*-test with *p* < 0.05 taken as significance level. Cytotoxicity of compounds **4d,i,l-n,p** was assessed by using KB cells, a cell line derived from a human carcinoma of the nasopharynx. All compounds tested showed low cytotoxicity in the MTT tests, thus demonstrating a good therapeutic index (>300).

5. In Vivo Antimalarial Activity. Compound **2** and CLT were tested in female CD1, 20–25 g mice infected with *P. berghei* ANKA, while compounds **4e–g,n**, together with **2** and CLT, were tested in mice infected with *P. chabaudi* AS. For both murine strains of *Plasmodium*, in vivo antimalarial activity evaluation was performed according to the four-day suppressive test of Peters et al.²⁹ Compounds were orally or intraperitoneally administered once daily for 4 days, with the first drug administered 2 h after i.v. parasite inoculation. Parasitaemia in untreated control mice generally reached 40% for *P. chabaudi* and 10% for *P. berghei*.

As displayed in Table 6, at an oral dose of 150 mg/kg, CLT only caused a 28% suppression of parasitaemia against *P. berghei*, and, to our surprise, one of the most active compounds in vitro against CQ-S strains, namely, **2**, was inactive at an oral dose of 30 mg/kg (data not shown) and only poorly active (37%) after i.p. administration, while the control drug CQ was able to suppress parasitaemia by 89% at a dose of 10 mg/kg. However, we found that **2**, but not CLT, was active in vivo against *P. chabaudi* with parasitaemia suppression of 94.8% after oral administration at a dose of 50 mg/kg. These rodent species notably differ in their ability to invade mature or immature erythrocytes and in their degrees of synchronism. At the moment, we have no explanation for the different activity of **2** against these strains, and further studies are in progress to address this question. However, it is of note that, in vitro, compound **2** and its analogues generally display higher IC₅₀ values against synchronous 3D7 and K1 strains than asynchronous D10 and W2 strains. Consequently, *P. chabaudi* was used for the further in vivo evaluation of **2** analogues, and both **4e** and **4n** demonstrated high antimalarial activity in vivo. Doses of 50 mg/kg achieved 94.0 and 99.4% of parasitaemia suppression, respectively. On the other hand, at the same dose, compound **4g** showed 73.1% parasitaemia suppression, complementing its lower in vitro antimalarial activity with respect to **4e** and **4n**.

Conclusions

This work led to the discovery of potent antimalarial agents structurally unrelated to known antiplasmodial drugs currently used in therapy or under development. The design strategy was focused on the development of polyaromatic compounds with high antimalarial activity especially against CQ-R strains and with high selectivity for free heme over P450 cytochrome prosthetic groups. The in vitro and in vivo antimalarial activity of this innovative class of antimalarials is dependent upon several key structural features: (i) the protonatable lateral chain, responsible for improved pharmacokinetic properties with respect to CLT, for selectivity for free heme over P450 cytochrome heme and for higher FV accumulation; (ii) the imidazole ring, with its dual role of heme-complexing group and substrate for the redox reaction generating the trityl radical; and (iii) the aryl/heteroaryl systems whose structural and electronic properties are key factors to influence the stabilization of a trityl radical intermediate.

Exploiting this novel pharmacophore potent antimalarials were identified, characterized by low in vitro toxicity against

different human and murine cell lines and with no activity against fungal pathogens.

The most promising antimalarial compounds **2**, **4e**, and **4n** were further evaluated to assess their efficacy in vivo and resulted in activity against *P. chabaudi* in CD1 mice after oral administration in the four-day suppressive test. The development of these compounds is characterized by a limited number of synthetic steps and by low cost of goods. The straightforward synthetic pathway was further simplified with compound **4e**, where the chiral center was removed thus avoiding racemate resolution and improving drugability.

In summary, both antimalarials **2** and **4e**, which combine low propensity to generate rapid resistance, potent in vitro and in vivo activity against various plasmodia, and endowed with oral bioavailability, prove to be promising candidates for early preclinical development. These novel compounds meet the requirements necessary for their potential development as antimalarials in poverty-stricken regions where oral bioavailability, stability of the formulation, and affordability are strict selection criteria beside activity against drug-resistant strains and rapid mode of action.

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. ¹H NMR and ¹³C NMR spectra were recorded on Bruker 200 MHz, Varian 300 MHz, or Bruker 400 MHz spectrometer 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 $\pm 0.4\%$ 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. All the organic layers were dried using anhydrous sodium sulfate.

($\pm$)-(3-Chlorophenyl)[4-(*N,N*-diethylaminomethyl)phenyl]methanol (7a**).** To a stirred suspension of magnesium turnings (122 mg, 5.0 mmol) in anhydrous tetrahydrofuran (THF; 8.0 mL), a solution containing *N*-(4-bromobenzyl)-*N*-ethylethanamine **6**³⁰ (1.32 g, 5.0 mmol) in dry THF (8.0 mL) was slowly added. The reaction mixture was stirred and heated under reflux until all magnesium was consumed (1–2 h). Thereafter, the reaction was cooled to 0 °C and a solution of 3-chlorobenzaldehyde **5** (506 mg, 3.6 mmol) in 10 mL of anhydrous THF was added during 2 h, maintaining the temperature between –5 and 0 °C. The reaction mixture was quenched by the dropwise addition of 20% ammonium chloride solution (30 mL). The layers were separated and the aqueous layer was extracted with EtOAc. The organic layer was washed with 10% Na₂CO₃ solution and dried over Drierite. Evaporation of the solvent afforded a residue, which was purified by flash chromatography (2% MeOH in DCM) to give **7a** as a light yellow oil (1.0 g, 92%): ¹H NMR (200 MHz, CDCl₃) δ 7.38–7.21 (m, 8H), 5.67 (s, 1H), 3.79 (bs, 1H), 3.52 (s, 2H), 2.47 (q, 4H, *J* = 7.1 Hz), 1.01 (t, 6H, *J* = 7.1 Hz); ESI MS *m/z* (*M* + *H*)⁺ 304.

($\pm$)-(3-Chlorophenyl)[4-(*N*-tert-butoxycarbonylpiperazin-4-yl)methyl]phenyl]methanone (9**).** To a stirred solution of **8**⁶ (1.21 g, 3.9 mmol) in dry MeCN (15 mL) at 0 °C was added a solution of *N*-Boc-piperazine (0.860 g, 4.6 mmol) and Et₃N (0.64 mL, 4.6 mmol) in MeCN (5 mL), and the reaction mixture was allowed to stir for 2 h. Thereafter, the reaction was quenched with H₂O and the solvent was evaporated under reduced pressure. The residue

was treated with water and extracted with CHCl₃. The combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated. The residue was chromatographed (1% MeOH in DCM) to afford **9** as a yellow oil (1.2 g, 74%): ¹H NMR (200 MHz, CDCl₃) δ 7.75–7.71 (m, 3H), 7.65–7.61 (m, 1H), 7.55–7.51 (m, 1H), 7.46–7.39 (m, 3H), 3.56 (s, 2H), 3.43 (t, 4H, *J* = 5.0 Hz), 2.39 (t, 4H, *J* = 4.9 Hz), 1.43 (s, 9H); ESI MS *m/z* (*M* + *H*)⁺ 415.

($\pm$)-(3-Chlorophenyl)[4-(*N*-tert-butoxycarbonylpiperazin-4-yl)methyl]phenyl]methanol (7b**).** To a solution of **9** (0.746 g, 1.8 mmol) in ethanol (5 mL), sodium borohydride (140 mg, 3.7 mmol) was added portionwise at 0 °C. The resulting mixture was stirred at rt for 30 min and then treated with H₂O and concentrated under reduced pressure. The residue was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated. The residue was chromatographed (2% MeOH in DCM) to afford **7b** as a yellow oil (540 mg, 72%): ¹H NMR (200 MHz, CDCl₃) δ 7.38–7.36 (m, 2H), 7.27–7.22 (m, 6H), 5.76 (s, 1H), 3.46 (s, 2H), 3.39 (t, 4H, *J* = 4.9 Hz), 2.34 (t, 4H, *J* = 5.0 Hz), 1.44 (bs, 1H), 1.42 (s, 9H); ESI MS *m/z* (*M* + *H*)⁺ 417.

7-Chloroquinoline-4-carbaldehyde (11b**).** A solid mixture of 4,7-dichloroquinoline **10** (2.97 g, 15.1 mmol) and [1,2-bis(diphenylphosphino)ethane]-dichloronickel(II) (60.0 mg, 0.114 mmol) was evacuated and flushed with argon several times. Dry diethylether (40 mL) was then added and the solution was stirred at 0 °C. Thereafter, a 3.0 M solution of methylmagnesium bromide in diethylether (5.0 mL, 15.1 mmol) was added dropwise. The reaction mixture was allowed to warm at rt and stirred for 24 h; thereafter, it was poured into saturated NH₄Cl solution, and the ether layer was separated, dried over Na₂SO₄, and evaporated under reduced pressure to afford a crude solid that was purified by flash chromatography (15% EtOAc in Hex) to afford 4-methyl-7-chloroquinoline as a white solid (1.5 g, 56%): mp 59–61 °C (lit. mp 57–58 °C).¹⁶ A stirred mixture of the above compound (1.17 g, 6.6 mmol) and selenium dioxide (1.17 g, 10.6 mmol) in bromobenzene (16 mL) was heated to 170 °C for 18 h. The selenium was filtered off and washed with DCM and the filtrate was evaporated to dryness. The residue was taken up in DCM, filtered through celite, and evaporated to dryness, affording **11b** as a colorless crystalline solid (740 mg, 57%): mp (EtOH) 108–110 °C (lit. mp 107–108 °C).³¹

($\pm$)-(3-Chlorophenylphenyl)(quinolin-4-yl)methanol (13**).** A solution of **11a** (0.487 g, 3.1 mmol) in dry THF (25 mL) was added dropwise to a solution of 3-chlorophenylmagnesium bromide (0.5 M in THF, 3.1 mmol, 6.2 mL), and the resulting solution was heated to 75 °C for 6 h. The reaction mixture was quenched with 20% ammonium chloride solution. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (2% MeOH in CHCl₃) to give **13** as a colorless oil (0.40 g, 48%): ¹H NMR (200 MHz, CDCl₃) δ 8.77 (d, 1H, *J* = 8.0 Hz), 8.10 (d, 1H, *J* = 6.0 Hz), 7.92 (d, 1H, *J* = 8.1 Hz), 7.68–7.45 (m, 3H), 7.40–7.12 (m, 4H), 6.39 (s, 1H), 3.55 (bs, 1H); ESI MS *m/z* (*M* + *H*)⁺ 270.

($\pm$)-[4-(Pyrrolidin-1-ylmethyl)phenyl](quinolin-4-yl)methanol (14a**).** To a stirred solution of **12** (0.624 g, 2.6 mmol) in dry THF (15 mL) was added dropwise *n*-butyllithium (1.6 M in Hex, 1.8 mL, 2.9 mmol) at –78 °C and was further stirred at –60 °C for 3 h. Thereafter, a solution of 4-quinolinecarbaldehyde (0.456 g, 2.9 mmol) in dry THF was added dropwise, and the temperature was maintained at –60 °C for 2 h. Subsequently, the reaction mixture was allowed to cool to rt, stirred for 24 h, and finally quenched with a saturated solution of NH₄Cl. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (6% MeOH in DCM) to afford **14a** as a brown oil (0.27 g, 32%): ¹H NMR (200 MHz, CDCl₃) δ 8.80 (d, 1H, *J* = 4.2 Hz), 8.06 (d, 1H, *J* = 8.2 Hz), 7.90 (d, 1H, *J* = 8.2 Hz), 7.68–7.60 (m, 2H), 7.56–7.29 (m, 1H), 7.24–7.20 (m, 4H), 6.41 (s, 1H), 3.71

(bs, 1H), 3.52 (s, 2H), 2.42 (m, 4H), 1.71 (m, 4H); ESI MS m/z (M + H)⁺ 319.

(±)-[4-(Pyrrolidin-1-ylmethyl)phenyl](7-chloroquinolin-4-yl)methanol (**14b**). Starting from **11b** (170 mg, 0.89 mmol), the title compound was prepared following the above-described procedure and was obtained as a brown oil (62.0 mg, 20%): ¹H NMR (200 MHz, CDCl₃) δ 8.87 (d, 1H, *J* = 4.5 Hz), 8.06 (d, 1H, *J* = 1.9 Hz), 7.83 (d, 1H, *J* = 9.0 Hz), 7.67 (d, 1H, *J* = 4.3 Hz), 7.38–7.25 (m, 5H), 6.36 (s, 1H), 3.68 (bs, 1H), 3.54 (s, 2H), 2.45 (m, 4H), 1.73 (m, 4H); ESI MS m/z (M + H)⁺ 353.

(±)-1-[(3-Chlorophenyl)[4-(*N,N*-diethylaminomethyl)phenyl]methyl]-1*H*-imidazole (**3a**). To a solution of **7a** (75.9 mg, 0.25 mmol) and a drop of DMF in dry DCM (10 mL) was added SOCl₂ (63.5 μL, 0.87 mmol) in dry DCM (2 mL) at 0 °C, and the mixture was stirred at the same temperature for 20 min and thereafter at room temperature for 3.5 h. The volatiles were removed, and the residue was washed with dry MeCN and concentrated under reduced pressure to remove residual SOCl₂. The resulting hydrochloride salt (89.7 mg, 0.25 mmol) was suspended in dry MeCN (5 mL) and to this suspension was slowly added a solution containing Et₃N (70.0 μL, 0.50 mmol) and imidazole (34.1 mg, 0.50 mmol) in MeCN (5 mL) at 0 °C. Thereafter, the reaction mixture was heated to 80 °C for 4 h. The solvent was evaporated under reduced pressure, and the residue was treated with H₂O and extracted with EtOAc. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by flash column chromatography (1% MeOH in DCM) to afford **3a** as a brown oil (60.2 mg, 68%): ¹H NMR (200 MHz, CDCl₃) δ 7.39–7.27 (m, 5H), 7.09–6.94 (m, 3H), 6.82 (s, 3H), 6.45 (s, 1H), 3.56 (s, 2H), 2.52 (q, 4H, *J* = 7.0 Hz), 1.03 (t, 6H, *J* = 7.0 Hz); MS m/z (M + H)⁺ 354. Anal. (C₂₁H₂₄ClN₃) C, H, N.

(±)-1-[(3-Chlorophenyl)[4-(*N,N*-diethylaminomethyl)phenyl]methyl]-1*H*-1,2,4-triazole (**3b**). Starting from **7a** (66.8 mg, 0.22 mmol), the title compound was prepared following the above-described procedure and was obtained as brown oil (52.0 mg, 68%): ¹H NMR (200 MHz, CDCl₃) δ 8.01 (s, 1H), 7.86 (s, 1H), 7.39–7.28 (m, 5H), 7.08–7.01 (m, 3H), 6.93–6.89 (m, 1H), 3.56 (s, 2H), 2.51 (q, 4H, *J* = 7 Hz), 1.03 (t, 6H, *J* = 6.9 Hz); ESI MS m/z (M + H)⁺ 355. Anal. (C₂₀H₂₃ClN₄) C, H, N.

(±)-1-[(3-Chlorophenyl)[4-[(*N*-tert-butoxycarbonyl)piperazin-4-ylmethyl]phenyl]methyl]-1*H*-imidazole (**3c**). Starting from **7b** (0.54 g, 1.3 mmol), the title compound was prepared following the above-described procedure and was obtained as brown oil (0.37 g, 61%): ¹H NMR (200 MHz, CDCl₃) δ 7.42 (s, 1H), 7.37–7.21 (m, 4H), 7.04–6.93 (m, 5H), 6.80 (s, 1H), 6.43 (s, 1H), 3.47 (s, 2H), 3.39 (t, 4H, *J* = 4.7 Hz), 2.35 (t, 4H, *J* = 4.7 Hz), 1.42 (s, 9H); ESI MS m/z (M + H)⁺ 467. Anal. (C₂₆H₃₁ClN₄O₂) C, H, N.

(±)-1-[(3-Chlorophenyl)[4-[(piperazin-1-yl)methyl]phenyl]methyl]-1*H*-imidazole (**3d**). A solution of **3c** (98.1 mg, 0.21 mmol) in a 1:1 TFA/DCM mixture (3.5 mL) was stirred at 0–5 °C for 3 h. Then the solvent was evaporated and the residue was treated with 2.5 M NaOH. The aqueous phase was extracted with DCM, and the organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified by flash column chromatography (8% MeOH in CHCl₃) to afford **3d** as a yellow oil (67.0 mg, 87%): ¹H NMR (200 MHz, CDCl₃) δ 7.37 (s, 1H), 7.32–7.22 (m, 4H), 7.08–6.91 (m, 5H), 6.81 (s, 1H), 6.43 (s, 1H), 3.47 (s, 2H), 2.87 (t, 4H, *J* = 4.7 Hz), 2.39 (t, 4H, *J* = 4.3 Hz), 2.22 (bs, 1H); ESI MS m/z (M + H)⁺ 367. Anal. (C₂₁H₂₃ClN₄) C, H, N.

(±)-4-[(1*H*-Imidazol-1-yl)[4-(3-chlorophenyl)methyl]quinoline (**3e**). Starting from **13** (0.38 g, 1.4 mmol), the title compound was prepared following the above-described procedure and was obtained as pale yellow oil (0.29 g, 65%): ¹H NMR (200 MHz, CDCl₃) δ 8.88 (d, 1H, *J* = 8.0 Hz), 8.14 (d, 1H, *J* = 6.0 Hz), 7.94 (d, 1H, *J* = 8.1 Hz), 7.68–7.45 (m, 3H), 7.43–7.10 (m, 6H), 6.89 (s, 1H), 6.74 (d, 1H, *J* = 4.4 Hz); ESI MS m/z (M + H)⁺ 320. Anal. (C₁₉H₁₄ClN₃) C, H, N.

(±)-4-[(1*H*-Imidazol-1-yl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl]quinoline (**3f**). To a solution of **14a** (0.382 g, 1.2 mmol) and a drop of DMF in dry DCM (5 mL) was added SOCl₂ (263 μL, 3.6 mmol) in dry DCM (5 mL) at 0 °C, and the mixture was

stirred at 0 °C for 20 min and thereafter at rt for 5 h. After evaporation of the solvent, the residue was dissolved in EtOAc and the organic layer was washed with saturated NaHCO₃, H₂O, and brine and dried over Na₂SO₄. The crude product was purified by flash chromatography (2% MeOH in DCM) to afford 4-[chloro[4-(pyrrolidin-1-ylmethyl)phenyl]methyl]quinoline as a yellow oil (0.33 g, 78%): ¹H NMR (200 MHz, CDCl₃) δ 8.92 (d, 1H, *J* = 4.3 Hz), 8.13 (d, 1H, *J* = 7.8 Hz), 7.96 (d, 1H, *J* = 8.0 Hz), 7.70–7.58 (m, 2H), 7.58–7.45 (m, 1H), 7.36–7.24 (m, 4H), 6.77 (s, 1H), 3.56 (s, 2H), 2.46 (m, 4H), 1.73 (m, 4H); ESI MS m/z (M + H)⁺ 337. To a stirred solution of the above compound (250 mg, 0.74 mmol) in dry DMF (1 mL) was added imidazole sodium salt (136 mg, 1.5 mmol), and the reaction mixture was heated to 80 °C for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with H₂O and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (2% MeOH in DCM) to give **3f** as a brown oil (140 mg, 52%): ¹H NMR (200 MHz, CDCl₃) δ 8.84 (d, 1H, *J* = 4.0 Hz), 8.14 (d, 1H, *J* = 8.2 Hz), 7.72–7.64 (m, 2H), 7.48–7.30 (m, 4H), 7.25–7.05 (m, 4H), 6.81 (s, 1H), 6.71 (d, 1H, *J* = 4.5 Hz), 3.58 (s, 2H), 2.47 (m, 4H), 1.75 (m, 4H); ESI MS m/z (M + H)⁺ 369. Anal. (C₂₄H₂₄N₄) C, H, N.

(±)-7-Chloro-4-[(1*H*-Imidazol-1-yl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl]quinoline (**3g**). Starting from **14b** (35.0 mg, 0.099 mmol), the title compound was prepared following the above-described procedure and was obtained as a brown oil (22.0 mg, 55%): ¹H NMR (200 MHz, CDCl₃) δ 8.85 (d, 1H, *J* = 4.0 Hz), 8.14 (s, 1H), 7.64 (d, 1H, *J* = 9.0 Hz), 7.42–7.32 (m, 4H), 7.25–7.04 (m, 4H), 6.81 (s, 1H), 6.69 (d, 1H, *J* = 4.3 Hz), 3.60 (s, 2H), 2.49 (m, 4H), 1.76 (m, 4H); ESI MS m/z (M + H)⁺ 403. Anal. (C₂₄H₂₃ClN₄) C, H, N.

(4-Chlorophenyl)[4-(diethylaminomethyl)phenyl]methanone (**16a**). To a stirred solution of **15** (3.5 g, 11.3 mmol) in dry MeCN (75 mL), cooled to 0 °C, diethylamine (1.41 mL, 16.9 mmol) and triethylamine (3.15 mL, 22.6 mmol) were added, and the resulting mixture was allowed to stir for 1 h at 0 °C. Thereafter, the reaction was quenched with H₂O and the solvent was evaporated under reduced pressure. The residue was treated with H₂O and extracted with CHCl₃. The combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated. The residue was chromatographed (2% MeOH in DCM) to afford **16a** as a yellow oil (2.8 g, 82%): ¹H NMR (300 MHz, CDCl₃) δ 7.74–7.70 (m, 4H), 7.47–7.41 (m, 4H), 3.62 (s, 2H), 2.53 (q, 4H, *J* = 7.0 Hz), 1.04 (t, 6H, *J* = 7.0 Hz); ESI MS m/z (M + H)⁺ 302.

(4-Chlorophenyl)[4-[(morpholin-4-yl)methyl]phenyl]methanone (**16b**). Starting from **15** (3.00 g, 9.7 mmol) and morpholine (1.02 mL, 11.6 mmol), the title compound was prepared following the above-described procedure and was obtained as a white low melting amorphous solid (2.5 g, 81%): ¹H NMR (300 MHz, CDCl₃) δ 7.76–7.72 (m, 4H), 7.47–7.44 (m, 4H), 3.74–3.71 (m, 4H), 3.57 (s, 2H), 2.48–2.45 (m, 4H); ESI MS m/z (M + H)⁺ 316.

(4-Chlorophenyl)[4-[(1-*tert*-butoxycarbonyl)piperazin-4-ylmethyl]phenyl]methanone (**16c**). Starting from **15** (2.88 g, 9.3 mmol) and *N*-Boc-piperazine (2.08 g, 11.2 mmol), the title compound was prepared following the above-described procedure and was obtained as a white low melting amorphous solid (2.0 g, 51%): ¹H NMR (300 MHz, CDCl₃) δ 7.73–7.69 (m, 4H), 7.43–7.40 (m, 4H), 3.55 (s, 2H), 3.43–3.40 (m, 4H), 2.40–2.37 (m, 4H), 1.42 (s, 9H); ESI MS m/z (M + H)⁺ 415.

(±)-4-(4-Chlorophenyl)[4-(diethylaminomethyl)phenyl]phenylmethanol (**17a**). The Grignard reagent was prepared in the usual manner as described for **7a** from bromobenzene (0.60 mL, 5.7 mmol), magnesium turnings (138 mg, 5.7 mmol), and a catalytic amount of iodine in dry THF (35 mL). A solution of **16a** (1.15 g, 3.8 mmol) in dry THF (25 mL) was added dropwise to the Grignard reagent, and the resulting solution was heated to 75 °C for 6 h. The reaction mixture was quenched with 20% ammonium chloride solution. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude residue

was purified by flash chromatography (2% MeOH in CHCl_3) to give **16a** as a yellow oil (220 mg, 45%): ^1H NMR (300 MHz, CDCl_3) δ 7.32–7.16 (m, 13H), 3.59 (m, 2H), 3.0 (bs, 1H), 2.55 (q, 4H, $J = 7.0$ Hz), 1.06 (t, 6H, $J = 7.0$ Hz); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 379.

($\pm$)-(4-Chlorophenyl)[4-[(morpholino-4-yl)methyl]phenyl]-phenylmethanol (**17b**). Starting from **16b** (0.347 g, 1.1 mmol), the title compound was prepared following the above-described procedure and was obtained as a yellow oil (0.35 g, 81%): ^1H NMR (300 MHz, CDCl_3) δ 7.29–7.18 (m, 13H), 3.64–3.61 (m, 4H), 3.46 (s, 2H), 2.41–2.38 (m, 4H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 394.

($\pm$)-(4-Chlorophenyl)[4-[1-(*tert*-butoxycarbonyl)piperazin-4-ylmethyl]phenyl]phenylmethanol (**17c**). Starting from **16c** (0.661 g, 1.6 mmol), the title compound was prepared following the above-described procedure and was obtained as a yellow oil (0.40 g, 51%): ^1H NMR (300 MHz, CDCl_3) δ 7.28–7.17 (m, 13H), 3.47 (s, 2H), 3.40–3.37 (m, 4H), 2.37–2.34 (m, 4H), 1.44 (s, 9H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 493.

($\pm$)-(4-Chlorophenyl)(4-fluorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methanol (**18a**). Starting from **16d** 6 (1.11 g, 3.7 mmol) and *p*-fluorobromobenzene, the title compound was prepared as described for **17a** and was obtained as a brown oil (0.94 g, 65%): ^1H NMR (300 MHz, CDCl_3) δ 7.33–7.16 (m, 10H), 7.01–6.99 (m, 2H), 3.66 (s, 2H), 2.78 (bs, 1H), 2.58 (m, 4H), 1.83–1.82 (m, 4H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 396.

(4-Chlorophenyl)bis[4-(pyrrolidin-1-ylmethyl)phenyl]methanol (**18b**). Starting from **16d** 6 (222 mg, 0.74 mmol) and **12**, 6 the title compound was prepared following the above-described procedure and was obtained as a brown oil (240 mg, 70%): ^1H NMR (300 MHz, CDCl_3) δ 7.27–7.15 (m, 12H), 3.59 (s, 4H), 3.59 (bs, 1H), 2.50–2.47 (m, 8H), 1.78–1.76 (m, 8H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 461.

(3-Chlorophenyl)(thien-2-yl)methanone (**20a**). To a stirred solution of 2-thiophenecarbaldehyde **19a** (2.38 g, 10.7 mmol) in dry THF (10 mL) was added dropwise 3-chlorophenylmagnesium-bromide (0.5 N solution in THF, 10.6 mL, 5.3 mmol), and the mixture was refluxed for 3 h. After cooling to rt, the reaction mixture was quenched by dropwise addition of a saturated solution of NH_4Cl . The aqueous phase was extracted with ethyl acetate and washed with brine. The combined organic layers were dried over Na_2SO_4 and evaporated. The crude residue was purified by flash chromatography (20% EtOAc in Hex) to afford the title compound as colorless prisms (0.99 g, 84%): mp (*n*-Hex) 95–96 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.82–7.80 (m, 1H), 7.75–7.70 (m, 2H), 7.63–7.61 (m, 1H), 7.56–7.52 (m, 1H), 7.45–7.42 (m, 1H), 7.18–7.15 (m, 1H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 223.

(3-Chlorophenyl)(thiazol-2-yl)methanone (**20b**). Starting from 3-chlorophenylmagnesium bromide and 2-thiazolecarbaldehyde **19b** (2.45 g, 22.1 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (2.3 g, 93%): mp (*n*-Hex) 87–88 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.41–8.39 (m, 2H), 8.03 (d, 1H, $J = 2.9$ Hz), 7.66 (d, 1H, $J = 2.9$ Hz), 7.47–7.44 (m, 2H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 224.

(3-Chlorophenyl)(furan-3-yl)methanone (**20c**). Starting from 3-chlorophenylmagnesium bromide and 3-furancarbaldehyde **19c** (1.47 g, 15.3 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (0.70 g, 45%): mp (*n*-Hex) 94–95 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.93 (dd, 1H, $J = 1.4$, 1.0 Hz), 7.83–7.81 (m, 1H), 7.74–7.71 (m, 1H), 7.58–7.54 (m, 1H), 7.53–7.51 (m, 1H), 7.46–7.40 (m, 1H), 6.89 (dd, 1H, $J = 1.7$, 1.0 Hz); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 207.

(3-Chlorophenyl)(pyridin-2-yl)methanone (**20d**). Starting from 3-chlorophenylmagnesium bromide and 2-pyridinecarboxaldehyde **19d** (1.00 g, 9.3 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (0.40 g, 40%): mp (*n*-Hex) 82–83 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.81–8.78 (m, 2H), 7.74–7.72 (m, 1H), 7.60–6.98 (m, 1H), 7.59–7.52 (m, 1H), 7.56–7.52 (m, 2H), 7.45–7.38 (m, 1H); MS m/z ($\text{M} + 1$) $^+$ 218; ESI MS m/z ($\text{M} + \text{H}$) $^+$.

(3-Chlorophenyl)(pyridyl-4-yl)methanone (**20e**). Starting from 3-chlorophenylmagnesium bromide and 4-pyridinecarboxaldehyde

19e (1.0 g, 9.3 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (0.63 g, 64%): mp (*n*-Hex) 99–100 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.82 (d, 1H, $J = 1.7$ Hz), 8.80 (d, 1H, $J = 1.5$ Hz), 7.79–7.77 (m, 1H), 7.67–7.64 (m, 1H), 7.62–7.58 (m, 1H), 7.56–7.54 (m, 2H), 7.47–7.41 (m, 1H); MS m/z ($\text{M} + \text{H}$) $^+$ 218.

(4-Chlorophenyl)(thien-2-yl)methanone (**21a**). Starting from 4-chlorophenylmagnesium bromide and 2-thiophenecarbaldehyde **19a** (1.98 g, 17.8 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (1.6 g, 80%): mp (*n*-Hex) 91–92 °C; ^1H NMR (CDCl_3) δ 7.84–7.79 (m, 2H), 7.74 (dd, 1H, $J = 1.1$ Hz, 4.7 Hz), 7.62 (dd, 1H, $J = 1.1$ Hz, 3.8 Hz), 7.49–7.45 (m, 2H), 7.16 (dd, 1H, $J = 3.8$ Hz, 4.7 Hz); ESI MS m/z ($\text{M} + 1$) $^+$ 223.

(4-Chlorophenyl)(thiazol-2-yl)methanone (**21b**). Starting from 4-chlorophenylmagnesium bromide and 2-thiazolecarbaldehyde **19b** (2.50 g, 22.1 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (2.3 g, 91%): mp (*n*-Hex) 79–80 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.44–8.41 (m, 2H), 7.98 (d, 1H, $J = 2.7$ Hz), 7.52 (d, 1H, $J = 3.0$ Hz), 7.45–7.42 (m, 2H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 224.

($\pm$)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thien-2-yl)methanol (**22a**). Starting from **12**, magnesium turnings, and **20a** (289 mg, 1.30 mmol), the title compound was prepared as described for **17a** and was obtained as colorless prisms (340 mg, 66%): mp (EtOAc/*n*-Hex) 70–71 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.43–7.42 (m, 1H), 7.38–7.22 (m, 8H), 6.96–6.93 (m, 1H), 6.72–6.71 (m, 1H), 3.73 (s, 2H), 3.10 (bs, 1H), 2.57–2.53 (m, 4H), 1.79–1.75 (m, 4H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 384.

($\pm$)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thiazol-2-yl)methanol (**22b**). Starting from **12**, magnesium turnings, and **20b** (1.21 g, 5.4 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (0.9 g, 46%): mp (EtOAc/*n*-Hex) 73–74 °C; ^1H NMR (200 MHz, CDCl_3) δ 7.81 (d, 1H, $J = 3.2$ Hz), 7.47 (s, 1H), 7.33 (d, 1H, $J = 3.2$ Hz), 7.31–7.25 (m, 7H), 4.24 (br, 1H), 3.62 (s, 2H), 2.54–2.49 (m, 4H), 1.80–1.76 (m, 4H); ESI-MS m/z ($\text{M} + \text{H}$) $^+$ 385.

($\pm$)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](furan-3-yl)methanol (**22c**). Starting from **12**, magnesium turnings, and **20c** (268 mg, 1.3 mmol), the title compound was prepared following the above-described procedure and was obtained as yellow prisms (300 mg, 26%): mp (EtOAc/*n*-Hex) 80–81 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.44–7.43 (m, 1H), 7.41–7.40 (m, 1H), 7.26 (m, 5H), 7.23–7.22 (m, 3H), 7.05 (dd, 1H, $J = 1.4$, 0.9 Hz), 6.29 (dd, 1H, $J = 1.6$, 0.9 Hz), 3.57 (s, 2H), 3.22 (br, 1H), 2.50–2.46 (m, 4H), 1.76–1.72 (m, 4H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 368.

($\pm$)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](pyridin-2-yl)methanol (**22d**). Starting from **12**, magnesium turnings, and **20d** (413 mg, 1.9 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (340 mg, 55%): mp (EtOAc/*n*-Hex) 68–69 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.56 (m, 1H), 7.65–7.59 (m, 1H), 7.37–7.01 (m, 10H), 6.36 (br, 1H), 3.60 (s, 2H), 2.52–2.48 (m, 4H), 1.78–1.74 (m, 4H); ESI-MS m/z ($\text{M} + \text{H}$) $^+$ 379.

($\pm$)-(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](pyridin-4-yl)methanol (**22e**). Starting from **12**, magnesium turnings, and **20e** (413 mg, 1.9 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (375 mg, 52%): mp (EtOAc/*n*-Hex) 75–76 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.44–8.42 (m, 2H), 7.33–7.20 (m, 7H), 7.16–7.09 (m, 3H), 3.62 (s, 2H), 2.56–2.52 (m, 4H), 1.80–1.76 (m, 4H); ESI MS m/z ($\text{M} + \text{H}$) $^+$ 379.

($\pm$)-(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thien-2-yl)methanol (**23a**). Starting from **12**, magnesium turnings, and **21a** (623 mg, 2.8 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (860 mg, 80%): mp (EtOAc/*n*-Hex) 69–70 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.35–7.34 (m, 1H), 7.32–7.31 (m, 1H), 7.28–7.24 (m, 7H), 6.92 (dd, 1H, $J = 3.5$, 4.9 Hz), 6.70 (dd, 1H, $J = 1.1$, 3.5

Hz), 4.39 (br s, 1H), 3.56 (s, 2H), 2.46–2.44 (m, 4H), 1.73–1.70 (m, 4H); ESI-MS m/z ($M + H$)⁺ 385.

(±)-(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thiazol-2-yl)methanol (**23b**). Starting from **12**, magnesium turnings, and **21b** (1.21 g, 5.4 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (1.1 g, 55%); mp (EtOAc/*n*-Hex) 78–79 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.70 (d, 1H, *J* = 3.2 Hz), 7.34–7.38 (m, 2H), 7.26–7.21 (m, 7H), 5.61 (br, 1H), 3.53 (s, 2H), 2.42 (m, 4H), 1.70 (m, 4H). ESI-MS m/z ($M + 1$)⁺ 385.

(±)-2-(7-Chloroquinolin-4-yl)-2-phenylacetonitrile (**25a**). A 60% oil dispersion of NaH (70.0 mg, 1.74 mmol) was washed with Hex and suspended in THF; thereafter, benzyliocyanide (0.17 mL, 1.5 mmol) in dry THF (10 mL) was added, and the mixture was heated under reflux for 30 min. Thereafter, compound **24** (0.15 g, 0.77 mmol) was added and the mixture was refluxed until completion. The solvent was evaporated under reduced pressure, the residue was treated with H₂O, neutralized with 5% acetic acid, and extracted with CHCl₃. The organic extracts were dried over Na₂SO₄, the solvent was removed, and the residue was purified by flash chromatography, eluting (15% EtOAc in Hex) to afford **25a** (0.18 g, 85%) as a viscous yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 8.93 (d, 1H, *J* = 4.5 Hz), 8.12 (d, 1H, *J* = 2.1 Hz), 7.81 (d, 1H, *J* = 8.7 Hz), 7.52–7.43 (m, 2H), 7.37–7.26 (m, 5H), 5.77 (s, 1H); ESI-MS m/z ($M + H$)⁺ 279.

(±)-2-(7-Chloroquinolin-4-yl)-2-(4-fluorophenyl)acetonitrile (**25b**). Starting from **24** (0.45 g, 2.3 mmol) and 4-fluorophenylacetonitrile (0.55 mL, 4.6 mmol), the title compound was prepared following the above-described procedure and was obtained as a viscous yellow oil (0.58 g, 85%); ¹H NMR (300 MHz, CDCl₃) δ 8.97 (d, 1H, *J* = 4.8 Hz), 8.17 (d, 1H, *J* = 2.4 Hz), 7.78 (d, 1H, *J* = 9.0 Hz), 7.53–7.49 (m, 2H), 7.33–7.26 (m, 2H), 7.11–7.04 (m, 2H), 5.75 (s, 1H); ESI-MS m/z ($M + H$)⁺ 297.

(7-Chloroquinolin-4-yl)phenylmethanone (**26a**). A 60% oil dispersion of NaH (0.31 g, 0.79 mmol) was washed with Hex and suspended in THF; thereafter, **25a** (0.220 g, 0.79 mmol) in dry THF (10 mL) was added at rt and stirred for 5 min, until evolution of H₂ ceased. A current of O₂ was passed into the yellow solution until the solution turned colorless. The solvent was evaporated under reduced pressure and residue was treated with H₂O and extracted with CHCl₃. The organic extracts were dried over Na₂SO₄, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (15% EtOAc in Hex) to afford **26a** as a viscous oil (0.20 g, 93%); ¹H NMR (300 MHz, CDCl₃) δ 8.98 (d, 1H, *J* = 6.3 Hz), 8.15 (d, 1H, *J* = 2.4 Hz), 7.81–7.78 (m, 3H), 7.64–7.58 (m, 1H), 7.48–7.42 (m, 3H), 7.37 (d, 1H, *J* = 4.2 Hz).

(7-Chloroquinolin-4-yl)(4-fluorophenyl)methanone (**26b**). Starting from **25b** (0.46 g, 1.56 mmol), the title compound was prepared following the above-described procedure and was obtained as a viscous oil (0.36 g, 90%); ¹H NMR (300 MHz, CDCl₃) δ 9.03 (d, 1H, *J* = 4.2 Hz), 8.20 (d, 1H, *J* = 2.4 Hz), 7.89–7.79 (m, 3H), 7.52–7.48 (m, 1H), 7.39 (d, 1H, *J* = 4.5 Hz), 7.19–7.13 (m, 2H).

(±)-[4-(Pyrrolidin-1-ylmethyl)phenyl](7-chloroquinolin-4-yl)phenylmethanol (**27a**). Starting from **12**, magnesium turnings, and **26a** (0.209 g, 0.78 mmol), the title compound was prepared as described for **17a** and was obtained as colorless prisms (0.11 g, 32%); mp (EtOAc/*n*-Hex) 124–125 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.64 (d, 1H, *J* = 4.8 Hz), 8.10 (d, 1H, *J* = 9.0 Hz), 8.02 (d, 1H, *J* = 2.4 Hz), 7.38–7.16 (m, 10H), 6.79 (d, 1H, *J* = 4.8 Hz), 3.98 (br, 1H), 3.61 (s, 2H), 2.52 (m, 4H), 1.80–1.75 (m, 4H); ESI MS m/z ($M + H$)⁺ 429.

(±)-(4-Fluorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](7-chloroquinolin-4-yl)methanol (**27b**). Starting from **12**, magnesium turnings, and **26b** (0.485 g, 1.7 mmol), the title compound was prepared following the above-described procedure and was obtained as colorless prisms (0.30 g, 40%); mp (EtOAc/*n*-Hex) 110–111 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.65 (d, 1H, *J* = 4.5 Hz), 8.06 (d, 1H, *J* = 9.3 Hz), 8.03 (d, 1H, *J* = 2.1 Hz), 7.32–6.98 (m, 9H), 6.78 (d, 1H, *J* = 4.8 Hz), 3.93 (bs, 1H), 3.61 (s, 2H), 2.52 (m, 4H), 1.79–1.77 (m, 4H); ESI MS m/z ($M + H$)⁺ 447.

(±)-1-[(4-Chlorophenyl)[4-(*N,N*-diethylaminomethyl)phenyl]phenylmethyl]-1*H*-imidazole (**4a**). To a solution of **17a** (323 mg, 0.85 mmol) and a drop of DMF in dry DCM (15 mL), cooled to 0 °C, was added SOCl₂ (180 μL, 2.5 mmol) in dry DCM (10 mL), and the mixture was stirred at 0 °C for 20 min and thereafter was heated to 45 °C for 4 h. The volatiles were removed and the residue was treated with dry MeCN and concentrated under reduced pressure to remove residual SOCl₂. The resulting hydrochloride salt (360 mg, 0.85 mmol) was suspended in dry MeCN (20 mL) and to this solution was slowly added a solution containing Et₃N (580 μL, 4.2 mmol) and imidazole (280 mg, 4.2 mmol) in MeCN (10 mL) at 0 °C. Thereafter, the reaction mixture was heated to 80 °C for 4 h. The solvent was evaporated under reduced pressure and the residue was treated with water and extracted with EtOAc. The combined organic extracts were dried over Na₂SO₄ and concentrated. The crude residue was purified by flash column chromatography (2% MeOH in CHCl₃) to afford **4a** as a yellow oil (30.0 mg, 26% yield); ¹H NMR (200 MHz, CDCl₃) δ 7.43 (s, 1H), 7.32–7.27 (m, 7H), 7.13–7.04 (m, 7H), 6.79 (s, 1H), 3.58 (s, 2H), 2.55 (q, 4H, *J* = 7.0 Hz), 1.05 (t, 6H, *J* = 7.0 Hz); ESI-MS m/z ($M - \text{imidazole}$)⁺ 364. Anal. (C₂₇H₂₈ClN₃) C, H, N.

(±)-1-[(4-Chlorophenyl)[4-(morpholin-4-ylmethyl)phenyl]phenylmethyl]-1*H*-imidazole (**4b**). Starting from **17b** (351 mg, 0.89 mmol), the title compound was prepared following the above-described procedure and was obtained as a light yellow viscous oil (260 mg, 66%); ¹H NMR (300 MHz, CDCl₃) δ 7.41 (s, 1H), 7.31–7.25 (m, 7H), 7.10–7.03 (m, 7H), 6.77 (m, 1H), 3.68 (m, 4H), 3.47 (s, 2H), 2.42 (m, 4H); ESI MS m/z ($M - \text{imidazole}$)⁺ 376. Anal. (C₂₇H₂₆ClN₃O) C, H, N.

(±)-1-[(4-Chlorophenyl)[4-(piperazin-4-ylmethyl)phenyl]phenylmethyl]-1*H*-imidazole (**4c**). Starting from **17c** (286 mg, 0.58 mmol), the title compound was prepared following the above-described procedure and was obtained as a brown amorphous solid (138 mg, 53%); ¹H NMR (300 MHz, CDCl₃) δ 7.40 (s, 1H), 7.30–7.24 (m, 8H), 7.09–7.01 (m, 6H), 6.76 (m, 1H), 3.46 (s, 2H), 2.84 (m, 4H), 2.38 (m, 4H), 1.73 (br, 1H); ESI MS m/z ($M - \text{imidazole}$)⁺ 375. Anal. (C₂₇H₂₇ClN₄) C, H, N.

(±)-1-[(4-Chlorophenyl)(4-fluorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]methyl]-1*H*-imidazole (**4d**). Starting from **18a** (0.348 g, 0.88 mmol), the title compound was prepared as described for **4a** and was obtained as brown oil (0.35 g, 88%); ¹H NMR (300 MHz, CDCl₃) δ 7.39 (s, 1H), 7.31–7.26 (m, 4H), 7.08–6.99 (m, 9H), 6.75 (s, 1H), 3.60 (s, 2H), 2.53–2.49 (m, 4H), 1.79–1.75 (m, 4H); ESI MS m/z ($M - \text{imidazole}$)⁺ 378. Anal. (C₂₇H₂₅ClFN₃) C, H, N.

1-[(4-Chlorophenyl)bis[4-(pyrrolidin-1-ylmethyl)phenyl]methyl]-1*H*-imidazole (**4e**). Starting from **18b** (143 mg, 0.31 mmol), the title compound was prepared following the above-described procedure and was obtained as brown oil (100 mg, 65%); ¹H NMR (200 MHz, CDCl₃) δ 7.40 (s, 1H), 7.31–7.25 (m, 6H), 7.06–7.01 (m, 8H), 3.62 (s, 4H), 2.53 (m, 8H), 1.78 (m, 8H); ESI MS m/z ($M - \text{imidazole}$)⁺ 444. Anal. (C₃₂H₃₅ClN₄) C, H, N.

(±)-1-[(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thien-2-yl)methyl]-1*H*-imidazole (**4f**). Starting from **22a** (0.345 g, 0.90 mmol), the title compound was prepared following the above-described procedure and was obtained as a brown oil (77.0 mg, 20%); ¹H NMR (300 MHz, CDCl₃) δ 7.40 (m, 1H), 7.37–7.30 (m, 4H), 7.27–7.25 (m, 1H), 7.13–7.12 (m, 1H), 7.09–7.07 (m, 2H), 7.06–7.05 (m, 1H), 7.03–6.99 (m, 2H), 6.88–6.85 (m, 2H), 3.63 (s, 2H), 2.54–2.50 (m, 4H), 1.81–1.77 (m, 4H); ESI MS m/z ($M - \text{imidazole}$)⁺ 366. Anal. (C₂₅H₂₄ClN₃S) C, H, N.

(±)-1-[(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thiazol-2-yl)methyl]-1*H*-imidazole (**4g**). Starting from **22b** (0.60 g, 1.56 mmol), the title compound was prepared following the above-described procedure and was obtained as a colorless oil (0.50 g, 74%); ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, 1H, *J* = 3.2 Hz), 7.55 (s, 1H), 7.37–7.23 (m, 5H), 7.11–6.94 (m, 6H), 3.63 (s, 2H), 2.58–2.45 (m, 4H), 1.87–1.76 (m, 4H); ESI-MS m/z ($M - \text{imidazole}$)⁺ 367. Anal. (C₂₄H₂₃ClN₄S) C, H, N.

($\pm$)-1-[(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](furan-3-yl)methyl]-1H-imidazole (**4h**). Starting from **22c** (0.184 g, 0.50 mmol), the title compound was prepared following the above-described procedure and was obtained as a colorless oil (42.0 mg, 20%): ^1H NMR (300 MHz, CDCl_3) δ 7.48–7.47 (m, 1H), 7.33–7.29 (m, 4H), 7.26 (m, 2H), 7.13 (m, 2H), 7.08 (m, 1H), 7.03 (m, 1H), 7.01 (m, 1H), 6.96–6.94 (m, 1H), 6.26 (s, 1H), 3.62 (s, 2H), 2.52 (m, 4H), 1.79 (m, 4H); ESI MS m/z ($\text{M} - \text{imidazole}$) $^+$ 350. Anal. ($\text{C}_{25}\text{H}_{24}\text{ClN}_3\text{O}$) C, H, N.

($\pm$)-1-[(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](pyridin-2-yl)methyl]-1H-imidazole (**4i**). Starting from **22d** (0.417 g, 1.1 mmol), the title compound was prepared following the above-described procedure and was obtained as a colorless oil (200 mg, 42%): ^1H NMR (300 MHz, CDCl_3) δ 8.69–8.66 (m, 1H), 7.69–7.63 (m, 1H), 7.58–7.57 (m, 1H), 7.31–7.23 (m, 5H), 7.10–7.04 (m, 3H), 6.99–6.91 (m, 4H), 3.60 (s, 2H), 2.52–2.48 (m, 4H), 1.79 (m, 4H); ESI-MS m/z ($\text{M} - \text{imidazole}$) $^+$ 361. Anal. ($\text{C}_{26}\text{H}_{25}\text{ClN}_4$) C, H, N.

($\pm$)-1-[(3-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](pyridin-4-yl)methyl]-1H-imidazole (**4j**). Starting from **22e** (0.379 g, 1.0 mmol), the title compound was prepared as described for **4a** and was obtained as a colorless oil (230 mg, 53%): ^1H NMR (300 MHz, CDCl_3) δ 8.63 (d, 2H, $J = 6.2$ Hz), 7.42–7.26 (m, 6H), 7.10–7.04 (m, 6H), 6.78 (s, 1H), 3.68 (s, 2H), 2.59 (m, 4H), 1.83 (m, 4H); ESI MS m/z ($\text{M} - \text{imidazole}$) $^+$ 361. Anal. ($\text{C}_{26}\text{H}_{25}\text{ClN}_4$) C, H, N.

($\pm$)-1-[(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thien-2-yl)methyl]-1H-imidazole (**4k**). Starting from **23a** (0.883 g, 2.3 mmol), the title compound was prepared following the above-described procedure and was obtained as a colorless oil (150 mg, 15%): ^1H NMR (200 MHz, CDCl_3) δ 7.40 (s, 1H), 7.34–7.27 (m, 5H), 7.10–7.04 (m, 5H), 7.00 (dd, 1H, $J = 3.8, 5.2$ Hz), 6.86 (dd, 1H, $J = 1.1, 3.8$ Hz), 6.85 (s, 1H), 3.63 (s, 2H), 2.55–2.50 (m, 4H), 1.81–1.77 (m, 4H); ESI-MS m/z ($\text{M} - \text{imidazole}$) $^+$ 366. Anal. ($\text{C}_{25}\text{H}_{24}\text{ClN}_3\text{S}$) C, H, N.

($\pm$)-1-[(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](thiazol-2-yl)methyl]-1H-imidazole (**4l**). Starting from **23b** (0.355 g, 1.0 mmol), the title compound was prepared following the above-described procedure and was obtained as a colorless oil (0.32 g, 72%): ^1H NMR (300 MHz, CDCl_3) δ 7.89 (d, 1H, $J = 3.4$ Hz), 7.53 (m, 1H), 7.36–7.26 (m, 5H), 7.08–6.98 (m, 6H), 3.58 (s, 2H), 2.48–2.44 (m, 4H), 1.75–1.71 (m, 4H); ESI-MS m/z ($\text{M} - \text{imidazole}$) $^+$ 367. Anal. ($\text{C}_{24}\text{H}_{23}\text{ClN}_4\text{S}$) C, H, N.

($\pm$)-1-[[4-(Pyrrolidin-1-ylmethyl)phenyl](7-chloroquinolin-4-yl)phenylmethyl]-1H-imidazole (**4m**). Starting from **27a** (90.1 mg, 0.21 mmol), the title compound was prepared as described for **4a** and was obtained as a brown oil (57.0 mg, 52%): ^1H NMR (300 MHz, CDCl_3) δ 8.87 (d, 1H, $J = 4.5$ Hz), 8.12 (d, 1H, $J = 2.4$ Hz), 7.39–7.07 (m, 13H), 6.87 (d, 1H, $J = 4.8$ Hz), 6.73 (s, 1H), 3.61 (s, 2H), 2.52 (m, 4H), 1.81–1.77 (m, 4H); ESI MS m/z ($\text{M} - \text{imidazole}$) $^+$ 411. Anal. ($\text{C}_{30}\text{H}_{27}\text{ClN}_4$) C, H, N.

($\pm$)-1-[(4-Fluorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl](7-chloroquinolin-4-yl)methyl]-1H-imidazole (**4n**). Starting from **27b** (0.380 g, 0.85 mmol), the title compound was prepared following the above-described procedure and was obtained as a brown amorphous solid (0.25 g, 60%): ^1H NMR (300 MHz, CDCl_3) δ 8.86 (d, 1H, $J = 4.8$ Hz), 8.10 (d, 1H, $J = 1.8$ Hz), 7.36–7.38 (m, 3H), 7.10–6.99 (m, 9H), 6.83 (d, 1H, $J = 4.8$ Hz), 6.70 (s, 1H), 3.60 (s, 2H), 2.50 (m, 4H), 1.77 (m, 4H); ESI MS m/z ($\text{M} - \text{imidazole}$) $^+$ 429. Anal. ($\text{C}_{30}\text{H}_{26}\text{ClFN}_4$) C, H, N.

($\pm$)-1-[(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]phenylmethyl]-1H-benzo[d]imidazole (**4o**). Starting from **17c**⁶ (94.0 mg, 0.22 mmol) and 1H-benzo[d]imidazole, the title compound was prepared following the above-described procedure and was obtained as brown oil (51.0 mg, 51%): ^1H NMR (200 MHz, CDCl_3) δ 7.83 (s, 1H), 7.77 (d, 1H, $J = 8.1$ Hz), 7.32–7.25 (m, 7H), 7.19–7.06 (m, 7H), 6.93–6.86 (m, 1H), 6.44 (d, 1H, $J = 8.3$ Hz), 3.59 (s, 2H), 2.50 (m, 4H), 1.81–1.76 (m, 4H); ESI MS m/z ($\text{M} - \text{benzimidazole}$) $^+$ 360. Anal. ($\text{C}_{31}\text{H}_{28}\text{ClN}_3$) C, H, N.

($\pm$)-1-[(4-Chlorophenyl)[4-(pyrrolidin-1-ylmethyl)phenyl]phenylmethyl]piperazine (**4p**). Starting from **17c**⁶ (94.0 mg, 0.22 mmol) and piperazine, the title compound was prepared following

the above-described procedure and was obtained as brown oil (51.0 mg, 51%): ^1H NMR (200 MHz, CDCl_3) 7.42–7.15 (m, 13H), 3.55 (s, 2H), 2.99 (m, 6H), 2.52 (m, 4H), 2.22 (m, 3H), 1.79–1.76 (m, 4H); ESI MS m/z ($\text{M} - \text{piperazine}$) $^+$ 360. Anal. ($\text{C}_{28}\text{H}_{32}\text{ClN}_3$) C, H, N.

1. 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, **2**, **3g**, **4q.r**, and the newly designed compounds (**3a–f** and **4a–p**) were calculated by using the ACD/ pK_a DB version 10.00 software (Advanced Chemistry Development Inc., Toronto, Canada), accordingly, percentage of neutral/ionized forms were computed at pH 7.4 (blood), 7.2 (cytoplasm), and 5.5 (*Pf* food vacuole) using the Handerson–Hasselbach equation.

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

Partial charges of the compounds, considered protonated at the imidazole and benzimidazole moieties as consequence of the estimation of apparent pK_a values, were assigned by comparing partial charges assigned by CFF91 force field³² with those calculated by MNDO³³ 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 field³²) by following our standard protocol. The system was heated up to 1000 K over 2000 fs (time step = 3.0); the temperature of 1000 K was applied to the system for 2000 fs (time step = 3.0), with the aim of surmounting torsional barriers; successively, temperature was linearly reduced to 300 K in 1000 fs (time step = 1.0). Resulting structures were subjected to energy minimization within Insight 2005 Discover 3 module (CFF91 force field,³² conjugate gradient algorithm,³⁴ $\epsilon = 80^*r$) 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 and (ii) torsional angles values, with the exception of those of lateral chains.

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 6.0 package³⁵ in Ampac/Mopac module of Insight 2000.1. GNORM value was set to 0.5. To reach a full geometry optimization, the criteria for terminating all optimizations was increased by a factor of 100 using the keyword PRECISE.

The dipole moments were calculated using partial charges obtained by the quantum mechanical method AM1 (Ampac/Mopac, Accelrys, San Diego) and visualized as vector (Decipher, Accelrys, San Diego). Connolly surfaces were computed and colored by AM1 charge distribution. LogD values of **2** and **4e** at pH 7.4 (blood), 7.2 (cytoplasm), and 5.5 (*Pf* FV) were calculated by using the ACD/ pK_a DB version 10.00 software (Advanced Chemistry Development Inc., Toronto, Canada).

Docking studies were carried out on CLT (global minimum conformer) into the homology model²⁶ of lanosterol 14 α -demethylase of *C. Albicans* using a docking methodology (Affinity, SA_Docking; Insight2005, Accelrys, San Diego) that considers all the systems (i.e., ligand and protein) flexible. Heme29.frc,²⁶ a forcefield including heme parameters, was used during all molecular simulations. Although in the subsequent flexible docking protocol all the systems were perturbed by means of Monte Carlo and simulated annealing procedures, nevertheless, the flexible docking procedure formally requires a reasonable starting structure. Accordingly, CLT was manually positioned into the active site, taking into account the binding mode of fluconazole (PDB code: 1EA1) and ketoconazole (PDB code: 1JIN) in cytochromes P450. The obtained complex was subjected to preliminary energy minimization (steepest descendent algorithm; $\epsilon = 80^*r$) until the maximum rms

derivative was less than 5 kcal/Å to generate roughly docked starting structure, as required by the Affinity docking procedure. Successively, flexible docking was achieved through the Affinity module in the Insight2005 suite, using the SA_Docking procedure³⁶ and using the Cell_Multipole³⁷ method for nonbond interactions. Lanosterol 14 α -demethylase binding domain area was defined as a flexible subset around the ligand containing all residues having at least one atom within a 6 Å radius from any given ligand atom. All atoms included in the binding domain area and the ligand were left free to move during the entire docking calculations, with the exception of the coordinating nitrogen, heme pyrroles, and the iron, which were tethered with a force constant of 30 kcal/Å². A Monte Carlo/minimization approach for random generation of a maximum of 20 structures was used, with an energy tolerance of 10⁶ kcal/mol to ensure a wide variance of the input structures to be minimized (2500 iterations; $\epsilon = 80^*r$). During this step, the ligand is moved by a random combination of translation, rotation, and torsional changes (Flexible_Ligand option, considering all rotatable bonds), to sample both the conformational space of the ligand and its orientation with respect to the enzyme. Van der Waals (vdW) and coulombic terms were scaled to a factor of 0.1 to avoid very severe divergences in the coulombic and vdW energies. The Metropolis test, at a temperature of 310 K, and a structure similarity check (rms tolerance = 0.3 kcal/Å) were applied to select acceptable structures. A total of 50 stages of simulated annealing (100 fs each) were applied on the resulting complexes. Over the course of the simulated annealing, system temperature was linearly decreased from 500 to 300 K; concurrently, the van der Waals and coulombic scale factors were similarly decreased from their initial values (defined above as 0.1) to their final value (1.0). A final round of 10⁵ minimization steps was applied at the end of the molecular dynamics. Resulting docked complexes were ranked by their conformational energy, and the geometry of imidazole-iron coordination bond. The coordination bond distances and angles were compared with those found by analyzing the iron-imidazole complexes found in CSDS (Cambridge Structural Database System, CSDS codes: CELWIZ, FEWXAG, KIWNIN, SIBZIM, SIBZOS, YILZIC, YILZOI, YIMYUO). The lowest energy complex showing the best coordination geometry was selected as representing structure for the most probable binding mode. Connolly surface was computed and colored on the basis of amino acids formal charge.

The experimentally determined structures of cytochromes P450 (PDB IDs: 1EA1, 1JIN, 1E9X, 1OXA, 1W0E, 1W0F, 1W0G) were downloaded from the Protein Data Bank (PDB; <http://www.rcsb.org/pdb/>). Hydrogens were added to all the PDB structures, considering a pH value of 7.2. P450 X-ray structures were superimposed by fitting heme atoms.

The sequences of cytochromes P450 were downloaded from the Sequence Retrieval System (<http://srs.embl-heidelberg.de:8000/srs5>). Sequence alignments were performed using ClustalW (WWW service at the European Bioinformatics Institute, <http://www.ebi.ac.uk/clustalw>) and Multiple_Alignment (Accelrys, San Diego). Results were analyzed using the Insight2005 Homology Module (Accelrys, San Diego).

2. In Vitro Antiplasmodial Activity Studies. D10 and W2 Strains. *P. falciparum* cultures were carried out according to Trager and Jensen's, with slight modifications.³⁸ 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; NaHCO₃ 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% O₂, 5% CO₂, 94% N₂. 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-bottom microplates (COSTAR) and serial dilutions were made. Asynchronous cultures with parasitaemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically

(OD650) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of the method of Makler in control and drug-treated cultures.³⁹ The antimalarial activity is expressed as 50% inhibitory concentrations (IC₅₀); each IC₅₀ value is the mean and standard deviation of at least three separate experiments performed in duplicate.

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 NaHCO₃, and Albu-MAXII (lipid-rich bovine serum albumin). All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO₂, 5% O₂, and 90% N₂. 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 performed on sterile 96-well microtiter plates, with each plate containing 100 μ L of the parasite culture (1% parasitaemia, 2.5% hemacrit). After 24 h of incubation at 37 °C, 3.7 Bq of [³H]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.⁴⁰

3. BHIA Assay. A total of 50 μ L of an 8 mM solution of hemin dissolved in DMSO was distributed in 96-well U-bottom microplates (0.4 μ mol/well; Costar 3799); 50 μ L of different compounds in water, in doses ranging from 1 to 8 mol equiv to hemin, was added to triplicate test wells. In control wells, 50 μ L of water was added. Water-insoluble compounds were solubilized in 25 μ L of DMSO and then added to hemin prepared at 16 mM and distributed into the wells in 25 μ L aliquots. The final concentration of DMSO/well was kept constant at 25%. β -Hematin formation was initiated by the addition of 100 μ L of 8 M acetate buffer (pH 5). Plates were incubated at 37 °C for 18 h to allow for complete reaction and then centrifuged at 3300g for 15 min. The soluble fraction of unprecipitated material was collected (fraction I). The remaining pellet was resuspended with 200 μ L of DMSO to remove unreacted hematin. Plates were then centrifuged again at 3300g for 15 min. The DMSO-soluble fraction (fraction II) was collected and the pellet, consisting of a pure precipitate of β -hematin, was dissolved in 0.1 M NaOH (fraction III) for spectroscopic quantitation. A 150 μ L aliquot of each fraction (I, II, or III) was transferred onto a new plate and serial 4-fold dilutions in 0.1 M NaOH were made. The amount of hematin was determined by measuring the absorbance at 405 nm using a microtiter plate reader (Molecular Devices). A standard curve of hematin dissolved in 0.1 M NaOH was used to calculate the amount of porphyrin present in each fraction. The drug concentration required to inhibit β -hematin formation by 50% (IC₅₀) was determined for each compound.²⁵

4. Cytotoxicity. Cell Lines. All cell lines were obtained from ATCC. The cells were cultured in RPMI 1640 supplemented with 5% FCS, 0.1 mM glutamine, 1% penicillin, and streptomycin. Cells were grown in Nunc clone plastic bottles (TedNunc, Roskilde, Denmark) and split twice weekly at different cell densities according to standard procedure. Perythelial blood mononuclear cells (MNC) were separated from heparinized whole blood obtained from a healthy donor on a Fycoll-Hypaque gradient. MNC thus obtained were washed twice with RPMI 1640 supplemented with 10% FCS, glutamine, and antibiotics, suspended at 200,000 viable cells/mL in medium containing, as mitogen, 5 μ g/mL PHA (Sigma) and used in toxicity tests.

Chemicals. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Aldrich. It was dissolved at a concentration of 5 mg/mL in sterile PBS at room temperature, and the solution was further sterilized by filtration and stored at 4 °C in a dark bottle. SDS was obtained from Sigma. Lysis buffer was prepared as follows: 20% w/v of SDS was dissolved at 37 °C in a solution of 50% of each DMF and demineralised water; pH was adjusted to 4.7 by adding 2.5% of an 80% acetic acid and 2.5% 1 N HCl solution.

Toxicity Experiments. Cells were plated at different concentrations on flat bottom 96-well microplates (0.1 mL/well). Lympho-

cytes were plated out at 20000 cells/well. NSO cells (plasmocytoma murine cell line) were plated out at 3000 cells/well, and Daudi cells (human lymphoblastoid cell line) were plated at 300 cells/well. A total of 12 h after plating, different concentrations of each compound dissolved in DMSO were added to each well. After 48 h, MTT assay was performed to analyze cytotoxicity of the different compounds. All experiments were performed at least two times in triplicate.

KB Cells. KB cells are a cell line derived from a human carcinoma of the nasopharynx, typically used as an assay for antineoplastic agents. KB cells are maintained as monolayers in RPMI 1640 + 10% HIFCS. All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO₂/95% air mixture. Drug toxicity assays: stock drug solutions were prepared in 100% DMSO (at 20 mg/mL, and ballmilled or sonicated if necessary). The stocks are kept at 4 °C. For the assays, the compound is further diluted to the appropriate concentration using complete medium. KB cells are harvested, counted, and washed in serum-free medium (2000 rpm, 10 min, 4 °C) and resuspended in fresh medium (RPMI 1640 + 10% HIFC) at a concentration of 4×10^4 /mL. A total of 100 μ L is added to wells on a 96-well plate (4×10^3 /well). The plate is incubated overnight at 37 °C, 5% CO₂/air mix, to allow the cells to adhere. Test compounds are prepared in 100% DMSO 20 mg/mL and diluted down to a starting concentration of 600 μ g/mL (2 $\times$ top concentration) with RPMI + 10% HIFCS. Control wells have no drug. A 10-fold serial dilution is performed across the plate, 300, 30, 3, and so on. Podophyllotoxin is the control drug. The plate is incubated for 72 h at 37 °C, 5% CO₂/air. Each well is assessed by microscope observation. A 20 μ L aliquot of Alamar Blue is then added to each well. Plates are incubated for a further 2–4 h before reading (Gemini), EX/EM 530/580, cutoff 550 nm. IC₅₀ (IC₉₀) values are calculated using sigmoidal regression analysis (MS *x*/fit).

MTT/Formazan Extraction Procedure. A total of 20 μ L of the 5 mg/mL stock solution of MTT was added to each well; after 2 h of incubation at 37 °C, 100 μ L of the extraction buffer was added. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured using a Titer-Tech 96-well multiscanner, employing the extraction buffer as the blank.

5. In Vivo Antiplasmodial Activity Studies. Briefly, blood is taken from donor mice and diluted to a parasitaemia of 1% (equivalent to 1×10^7 infected erythrocytes/mL). An inoculum of 0.2 mL is administered to each mouse by the intravenous route. At 2–4 h, post-inoculation dosing commences. Experimental compounds were prepared in 10% Tween-80/EtOH and sterile PBS. Insoluble formulations were ball-milled and placed into a sonicating waterbath, 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 p.o. every day for 4 days at 10 mg/kg, giving >90% parasite clearance on day 4. On day five post-infection, blood smears of all animals (2 slides/mouse) were prepared, fixed with methanol, and stained with 10% Giemsa stain. Percentage parasitaemia was determined microscopically. Results are reported as % infected erythrocytes and compared to the CQ control group and the untreated control group.

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Supporting Information Available: Antifungal activity for compounds **4e**, **n**, **2**, and **1** (Table 1), the corresponding experimental details, and elemental analyses for compounds **3a–g** and **4a–p** (Table 2). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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