

# N-Cinnamoylated Chloroquine Analogues as Dual-Stage Antimalarial Leads

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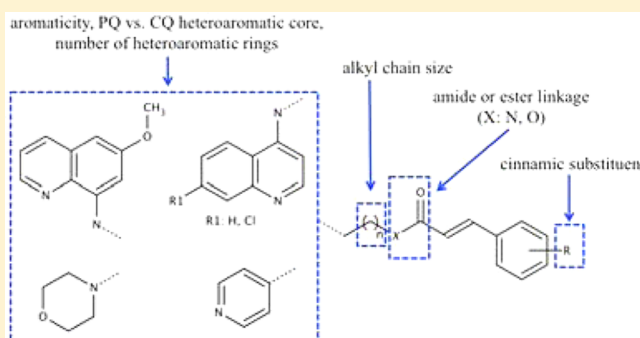
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## S Supporting Information

**ABSTRACT:** The control of malaria is challenged by drug resistance, and new antimalarial drugs are needed. New drug discovery efforts include consideration of hybrid compounds as potential multitarget antimalarials. Previous work from our group has demonstrated that hybrid structures resulting from cinnamic acid conjugation with heterocyclic moieties from well-known antimalarials present improved antimalarial activity. Now, we report the synthesis and SAR analysis of an expanded series of cinnamic acid derivatives displaying remarkably high activities against both blood- and liver-stage malaria parasites. Two compounds judged most promising, based on their in vitro activity and druglikeness according to the Lipinski rules and Veber filter, were active in vivo against blood-stage rodent malaria parasites. Therefore, the compounds reported represent a new entry as promising dual-stage antimalarial leads.



## INTRODUCTION

Several concerted efforts to control malaria and achieve its eradication have been made through history.<sup>1</sup> Despite initial success, with regional elimination in Southern Europe and some countries in North Africa and the Middle East, malaria has not been controlled in many other countries because of a number of factors.<sup>2</sup> Two main reasons for malaria remaining such a burden to humanity are the widespread resistance of the malaria parasite to available drugs and its complex life cycle.<sup>2</sup> Human malaria is triggered by infection by an intracellular *Plasmodium* parasite, which is transmitted to human hosts via an infected female *Anopheles* mosquito bite. Once in the host circulation, *Plasmodium* sporozoites initiate the asexual phase of the life cycle by quickly moving to the liver. During this clinically silent liver-stage infection, thousands of merozoites are formed and released into the bloodstream, where they invade red blood cells, thus starting the pathogenic asexual erythrocytic cycle, resulting in the various clinical manifestations of malaria.<sup>3</sup>

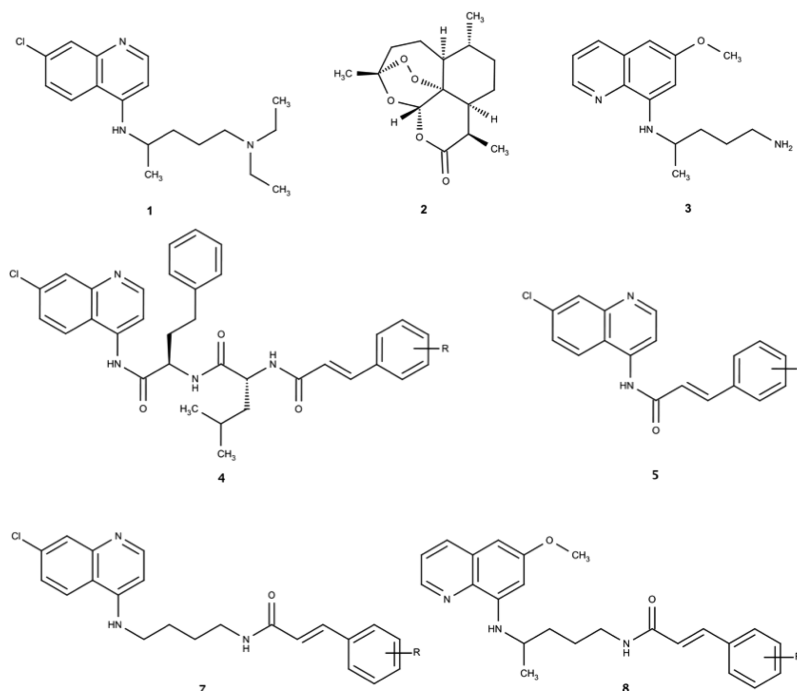
Resistance of malaria parasites to previous generations of medicines, such as chloroquine (**1**), became widespread in the 1970s and 1980s, undermining malaria control efforts. During the past decade, artemisinin-based combination therapies

(ACTs) have been adopted globally as the first line of treatment. ACTs include a rapidly acting artemisinin component and a partner drug to improve efficacy and hinder emergence of resistance to artemisinins.<sup>4</sup> However, there are now worrisome signs that malarial parasites are developing resistance to artemisinins (**2**),<sup>5</sup> and so the dependence of all new drug combinations on artemisinin derivatives is a matter of concern. While most available antimalarial agents target the blood-stage parasites, relatively few drugs are known to inhibit liver stage parasites. Primaquine (**3**) remains the only drug in clinical use worldwide that acts against liver stages of all *Plasmodium* species,<sup>6</sup> including *P. vivax* and *P. ovale* hypnozoites, which are latent forms unique to these species that cause relapsing malaria long after the original infection, complicating control efforts.<sup>7,8</sup> Taking into account concerns of drug resistance and difficult-to-treat chronic liver stages, optimal therapy should be effective against multidrug-resistant parasite strains and be active against both liver and erythrocytic parasite stages.

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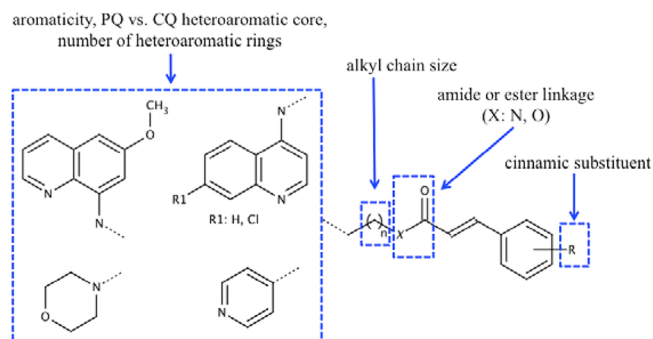




**Figure 1.** Chemical structures of chloroquine (1), artemisinin (2), primaquine (3), and first- (4 and 5) and second- (7 and 8) generation heterocycle-cinnamic acid conjugates.

A recent approach in antimalarial drug design characterized as “covalent bitherapy” involves linking two active molecules, thus packaging dual activity into a single hybrid molecule with potential to enhance efficacy, improve safety, and/or reduce propensity to elicit resistance relative to the parent drugs.<sup>9,10</sup> As part of our efforts to develop novel hybrids, we previously reported a first generation of heterocycle-cinnamic acid conjugates (4 and 5, Figure 1) linking the heteroaromatic ring of known antimalarials to a *trans*-acid cinnamic motif, active in vitro against blood-stage *P. falciparum*.<sup>11</sup> From these studies we discovered that a spacer between the heterocycle and the cinnamoyl moiety is required for antiparasmodial activity and that the higher the lipophilicity of conjugates 4, the higher is their in vitro antiparasmodial activity. On the basis of these results, we developed a second generation of conjugates (7 and 8, Figure 1) where the cinnamoyl core was linked to the heterocyclic core of known antimalarials through a flexible and more hydrophobic butylamine chain.<sup>12,13</sup> Compounds 7 ( $IC_{50}$  against cultured malaria parasites of 11–59 nM)<sup>13</sup> and 8 ( $IC_{50}$  = 1.4–2.4  $\mu$ M)<sup>12</sup> had higher in vitro potency than their parent compounds 1 ( $IC_{50}$  = 138 nM) and 3 ( $IC_{50}$  = 7.5  $\mu$ M), against blood-stage *P. falciparum* (W2 strain) and liver-stage *P. berghei*. These findings established the cinnamoyl core as a valuable pharmacophore to enhance potency of established antimalarials.

Building on these compounds, we have performed further SAR studies and herein report the synthesis and antimalarial assessment of a series of new heterocycle-cinnamic conjugates (in brief, HEFLECINs). In terms of SAR, the presence of aromaticity in the heterocyclic core, the number of the heteroaromatic rings, the length of the aliphatic chain, the nature (amide or ester) of the linkage between the heterocycle and cinnamic core, and the nature of the cinnamic substituent were all evaluated (Figure 2). Compounds were tested in vitro against erythrocytic stages of the human parasite *P. falciparum*; two of them, 7d and 7h, were further confirmed to be active in



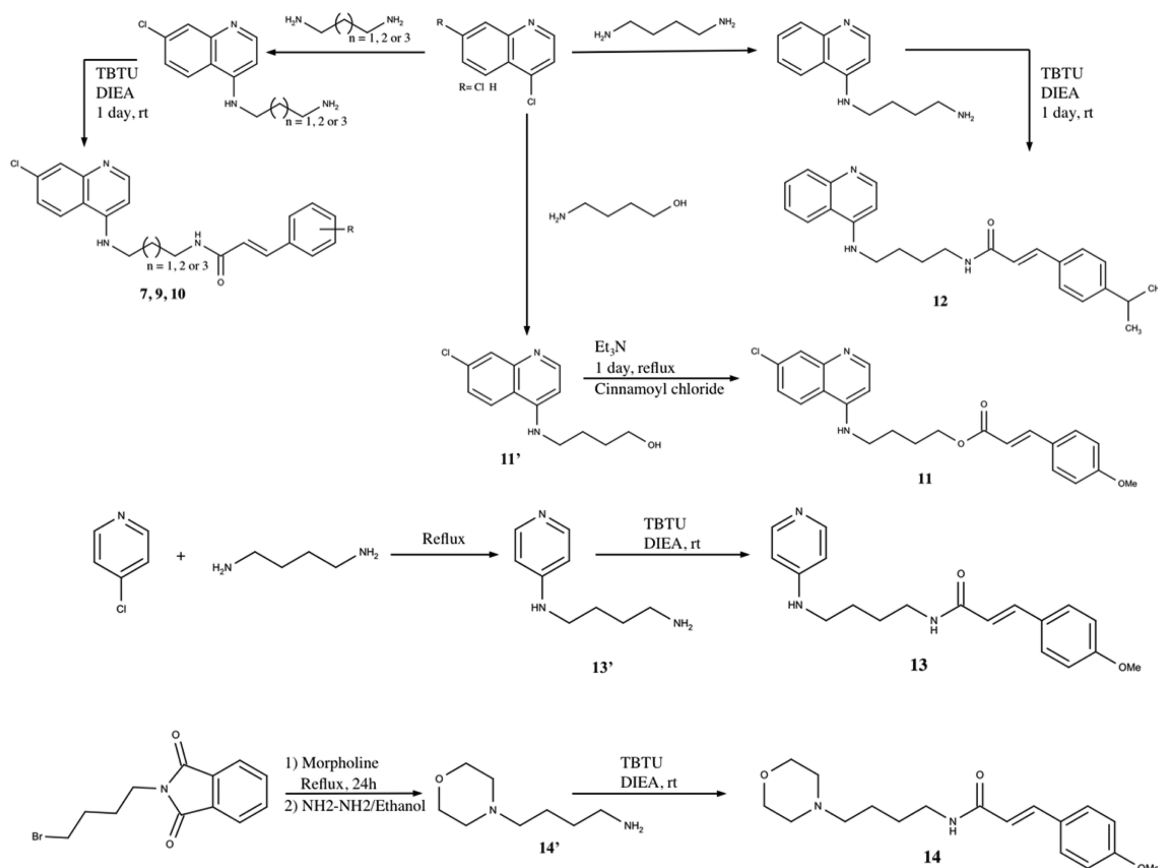
**Figure 2.** Structural parameters considered in the SAR analysis of the heterocycle-cinnamic acid conjugates.

vivo against the clinically relevant blood-stage infection in a *P. berghei* rodent malaria model. Additionally, our recent findings on the improved liver-stage antimalarial activity of *N*-cinnamoyl derivatives of primaquine<sup>12</sup> prompted us to evaluate the activity of heterocycle-cinnamic conjugates against liver-stage *P. berghei* parasites. Most of the HEFLECINs were structurally related to chloroquine, presented outstanding activity against both erythrocytic and liver stages of the parasite life cycle, and were noncytotoxic against Huh7 human hepatoma cells. To the best of our knowledge, chloroquine-related compounds active against both liver- and blood-stage parasites have not previously been reported. These results bring new insights into the development of dual action antimalarial agents.

## RESULTS AND DISCUSSION

**Chemistry.** For the past decade we have been working on the design, synthesis, and evaluation of potential antimalarial candidates, bearing in mind that synthetic pathways should be kept as cheap and straightforward as possible. The HEFLECINs reported here are examples of how simple chemistry can yield promising antimalarial leads (Scheme 1).

Scheme 1. Synthetic Pathways toward Heterocycle–Cinnamic Acid Conjugates 7 and 9–12



Compound **8d** was obtained through a single amide coupling step between the parent drug PQ (**3**) and the desired cinnamic acid, using previously reported standard peptide coupling conditions,<sup>12</sup> namely, activation of the cinnamic acid with *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) in the presence of *N*-ethyl-*N,N*-diisopropylamine (DIEA), followed by addition of PQ. Compounds **7**<sup>13</sup> and **9–13** were obtained in two steps, starting from (i) a classical nucleophilic aromatic substitution reaction between the appropriate diaminoalkane (1,3-diaminopropane, 1,4-diaminobutane, or 1,5-diaminopentane) or 4-aminobutan-1-ol (in the synthesis of **11**) and the desired chlorinated heterocycle (4,7-dichloroquinoline, 4-chloroquinoline, or 4-chloropyridine) followed by (ii) coupling the resulting compounds to the appropriate cinnamic acids, again conveniently activated by TBTU/DIEA. The synthesis of **14** was the only one requiring three steps, as (i) morpholine was first reacted with 1-(*N*-phthaloyl)amino-4-bromobutane followed by (ii) hydrazinolysis of the phthaloyl amino-protecting group to afford the free amine for subsequent (iii) PyBOP/DIEA-mediated coupling to the appropriate cinnamic acid.

Although not all synthesis yields were high, the rather small number of synthesis steps, cheap starting materials, and straightforward purification techniques (cf. Experimental Section) largely compensate for low yields. All the target compounds were obtained in high purity, as determined by high performance liquid chromatography with diode array detection (HPLC-DAD), and their structures were confirmed by proton (<sup>1</sup>H) and carbon-13 (<sup>13</sup>C) nuclear magnetic resonance (NMR), as well as by mass spectrometry (MS) analysis with electrospray ionization and ion-trap quadrupole

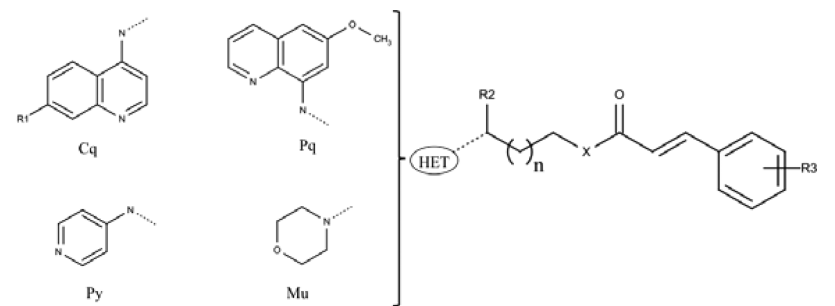
detection (ESI-IT). Relevant data are presented in the Experimental Section.

**Biology.** Our recent discovery of the potent in vitro activity of CQ analogues **7** against blood-stage CQ-resistant *P. falciparum* parasites unveiled the key role of the cinnamoyl moiety in reversing parasite resistance to CQ.<sup>13</sup> We have now expanded the family of heterocycle–cinnamic acid conjugates (HEFLECINs) and tested their in vitro activity against blood-stage *P. falciparum* (Table 1) in order to (i) establish relevant SAR (Figure 2) and (ii) select the most promising candidates for in vivo challenge in the *P. berghei* rodent malaria model. For some of the compounds we compared activity against chloroquine-sensitive (3D7) and -resistant (W2) *P. falciparum* parasites. Furthermore, given their structural analogy to CQ, which acts via inhibition of  $\beta$ -hematin formation, selected compounds were evaluated as in vitro inhibitors of this process (Table 1).<sup>14,15</sup> Finally, on the basis of our latest disclosure of primaquine–cinnamic acid conjugates (PRIMACINs) with improved in vitro activity against liver-stage *Plasmodia*,<sup>12</sup> we also assessed the in vitro activity of a subset of the HEFLECINs against liver-stage *P. berghei* (Figure 3, Table 1).

**In Vitro Activity against Intraerythrocytic *P. falciparum* Parasites.** Blood-stage activity of HEFLECINs was determined by methods previously reported by us.<sup>16,17</sup> Close inspection of our data (Table 1, columns 8 and 9) shows the following.

(i) Remarkably, most of the test compounds assayed against both strains were more active against the CQ-resistant (W2) strain than against the CQ-sensitive (3D7) strain.

(ii) Compounds from the **7**, **9**, and **10** series with a single substituent (R) on the cinnamoyl group (i.e., all except **7p**)

Table 1. In Vitro Data on Test Compounds for  $\beta$ -Hematin Inhibition, and Blood- and Liver-Stage Activity


The figure shows the chemical structures of the HET (4-hydroxy-2-methyl-6-methyl-2H-pyridine-3(1H)-one) and the four heterocyclic cores: Cq (4-chloroquinoline), Pq (4-methoxyquinoline), Py (pyridine), and Mu (morpholine). The general structure of the test compounds is shown as a central core (HET, Cq, Pq, Py, or Mu) connected via a polymethylene spacer (CH<sub>2</sub>)<sub>n</sub> to a cinnamoyl group (CH=CH-C(=O)-C<sub>6</sub>H<sub>4</sub>-R<sub>3</sub>), where R<sub>2</sub> is a substituent on the polymethylene spacer and X is a linker (O or S).

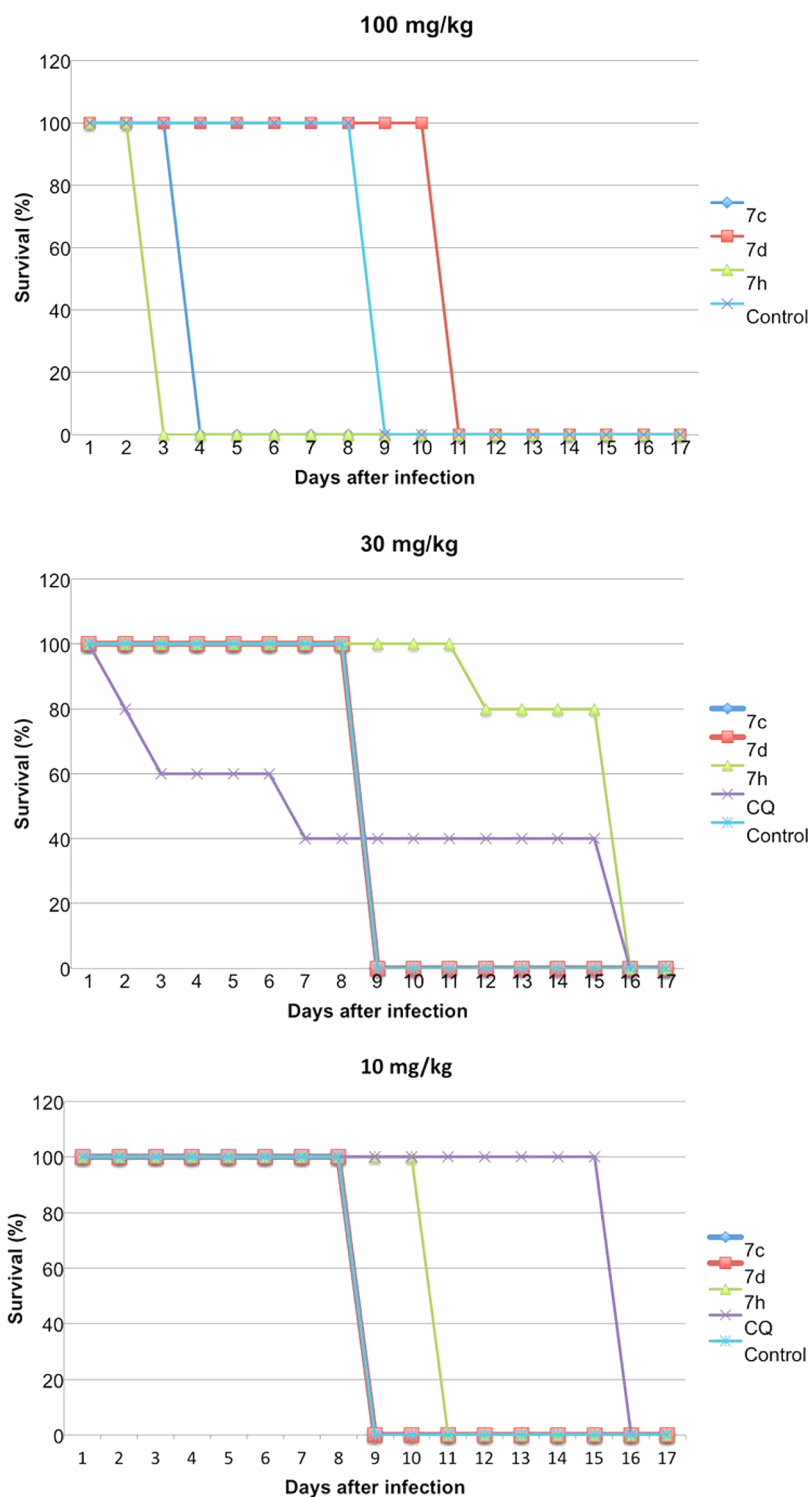
compd	HET	n	X	R <sub>2</sub>	R <sub>3</sub>	$\beta$ -hematin inhibition <sup>a</sup>	Pf W2 IC <sub>50</sub> (nM) <sup>b</sup>	Pf 3D7 IC <sub>50</sub> (nM) <sup>b</sup>	liver stage IC <sub>50</sub> ( $\mu$ M) <sup>c,g</sup>
7a	Cq R1 = Cl	2	N	H	H	NA	46.6 $\pm$ 5.5	62.85 $\pm$ 3.5	1.06 $\pm$ 0.1
7b					p-Me	NA	16.9 $\pm$ 1.2	24.23 $\pm$ 0.2	2.36 $\pm$ 0.5
7c					p- <sup>i</sup> Pr <sup>c</sup>	+	11.0 $\pm$ 6.2	15.63 $\pm$ 5.4	2.50 $\pm$ 0.2
7d					p-OMe <sup>c</sup>	+	20.0 $\pm$ 2.6	30.63 $\pm$ 9.5	2.86 $\pm$ 0.4
7e					p-NH <sub>2</sub>	NA	58.8 $\pm$ 1.5	78.12 $\pm$ 2.2	ND
7f					m-F	+	34.1 $\pm$ 4.2	50.92 $\pm$ 1.0	1.13 $\pm$ 0.2
7g					p-F	+	19.7 $\pm$ 0.4	39.85 $\pm$ 3.5	1.42 $\pm$ 0.2
7h					p-Cl <sup>d</sup>	+	11.6 $\pm$ 0.4	26.21 $\pm$ 2.0	1.44 $\pm$ 0.3
7i					p-Br	++	18.2 $\pm$ 2.8	28.75 $\pm$ 6.3	2.28 $\pm$ 0.6
7j					o-NO <sub>2</sub>	+	38.3 $\pm$ 4.2	56.91 $\pm$ 5.1	1.09 $\pm$ 0.2
7k					m-NO <sub>2</sub>	NA	26.2 $\pm$ 5.0	42.06 $\pm$ 7.1	ND
7l					p-NO <sub>2</sub>	++	23.5 $\pm$ 1.3	48.94 $\pm$ 3.2	ND
7o					p-NMe <sub>2</sub>	+	25.8 $\pm$ 2.1	ND	ND
7p					m,p-di-OMe	NA	110.8 $\pm$ 13.0	ND	4.05 $\pm$ 0.2
8d	Pq	2	N	CH <sub>3</sub>	p-OMe		>10000 <sup>h</sup>		2.35 $\pm$ 0.19 <sup>h</sup>
9a	Cq R1 = Cl	1	N	H	H	NA	74.8 $\pm$ 2.0	141.3 $\pm$ 9.3	ND
9b					p-Me	NA	47.4 $\pm$ 1.2	55.85 $\pm$ 5.1	ND
9c					p- <sup>i</sup> Pr	NA	38.0 $\pm$ 11.2	50.27 $\pm$ 1.0	ND
9d					p-OMe	NA	55.1 $\pm$ 2.3	49.51 $\pm$ 5.8	4.02 $\pm$ 0.6
9e					p-NH <sub>2</sub>	NA	79.8 $\pm$ 2.0	134.85 $\pm$ 5.4	ND
9f					m-F	NA	73.3 $\pm$ 6.8	137.95 $\pm$ 1.2	ND
9g					p-F	NA	46.7 $\pm$ 3.3	47.73 $\pm$ 1.3	ND
9h					p-Cl	+	41.6 $\pm$ 2.9	46.47 $\pm$ 4.5	ND
9i					p-Br	NA	38.2 $\pm$ 6.9	49.96 $\pm$ 3.5	ND
9j					o-NO <sub>2</sub>	+	52.1 $\pm$ 4.0	64.15 $\pm$ 6.7	ND
9k					m-NO <sub>2</sub>	+	50.3 $\pm$ 1.2	52.73 $\pm$ 5.3	ND
9l					p-NO <sub>2</sub>	+	23.7 $\pm$ 2.1	52.53 $\pm$ 10.8	ND
10a	Cq R1 = Cl	3	N	H	p- <sup>i</sup> Pr	+	31.5 $\pm$ 3.8	ND	ND
10b					p-OMe	NA	50.3 $\pm$ 2.6	ND	2.27 $\pm$ 0.6
10c					p-Cl	NA	25.1 $\pm$ 0.2	ND	1.62 $\pm$ 0.1
11	Cq R1 = Cl	2	O	H	p-OMe	+	121.9 $\pm$ 5.5	ND	6.47 $\pm$ 0.9
12	Cq R1 = H	2	N	H	p- <sup>i</sup> Pr	NA	114.9 $\pm$ 3.0	ND	ND
13	Py	2	N	H	p-OMe	NA	>10000	ND	>10
14	Mu	2	N	H	p- <sup>i</sup> Pr	NA	>10000	ND	>10
ART						—	9.5 $\pm$ 1.9	23.5 $\pm$ 0.8	ND
CQ						++	138 <sup>e</sup>	52.45	15.9 $\pm$ 0.0
PQ							3300 <sup>f</sup>		7.5 <sup>h</sup>

<sup>a</sup>Ability of the test compounds to inhibit hemozoin formation in vitro was calculated as a percent of the inhibitory effect displayed by reference drug CQ in the same experiment. Test compounds were ranked as follows: <50%, not active (—); between 50% and 75%, moderately active (+);  $\geq$ 75%, highly active (++) . <sup>b</sup>Blood-stage antiparasitic activity was determined against the CQ-resistant *P. falciparum* strain W2 and strain 3D7, using artemisinin (ART) and CQ as reference drugs. <sup>c</sup>IC<sub>50</sub> of the most active compounds against liver stage. <sup>d</sup>Also tested in vivo. <sup>e</sup>Value taken from ref 24. <sup>f</sup>Value taken from ref 17. <sup>g</sup>ND, not determined. <sup>h</sup>Value taken from ref 12.

were highly active, with IC<sub>50</sub> in the range 11–79 nM. These series differ only in the length of the polymethylene spacer between the chloroquinoline and cinnamoyl cores. Comparison between homologues from series 7, 9, and 10 (i.e., 7c vs 9c vs 10a, or 7h vs 9h vs 10c, or 7d vs 9d vs 10b) shows that a butyl spacer is preferred over pentyl and propyl spacers.

(iii) Removal of the 7-chlorine substituent from the chloroquinoline ring leads to a decrease in antimalarial activity of about 1 order of magnitude, as shown for compounds 7c (IC<sub>50</sub> = 11 nM) and its dechlorinated analogue 12 (115 nM).

(iv) Replacement of the amide bond in 7d (IC<sub>50</sub> = 20 nM) by its ester isostere, to afford 11, leads to a 6-fold decrease in activity (122 nM).



**Figure 3.** Survival curves for *P. berghei* infected mice treated with compounds 7c, 7d, and 7h and CQ.

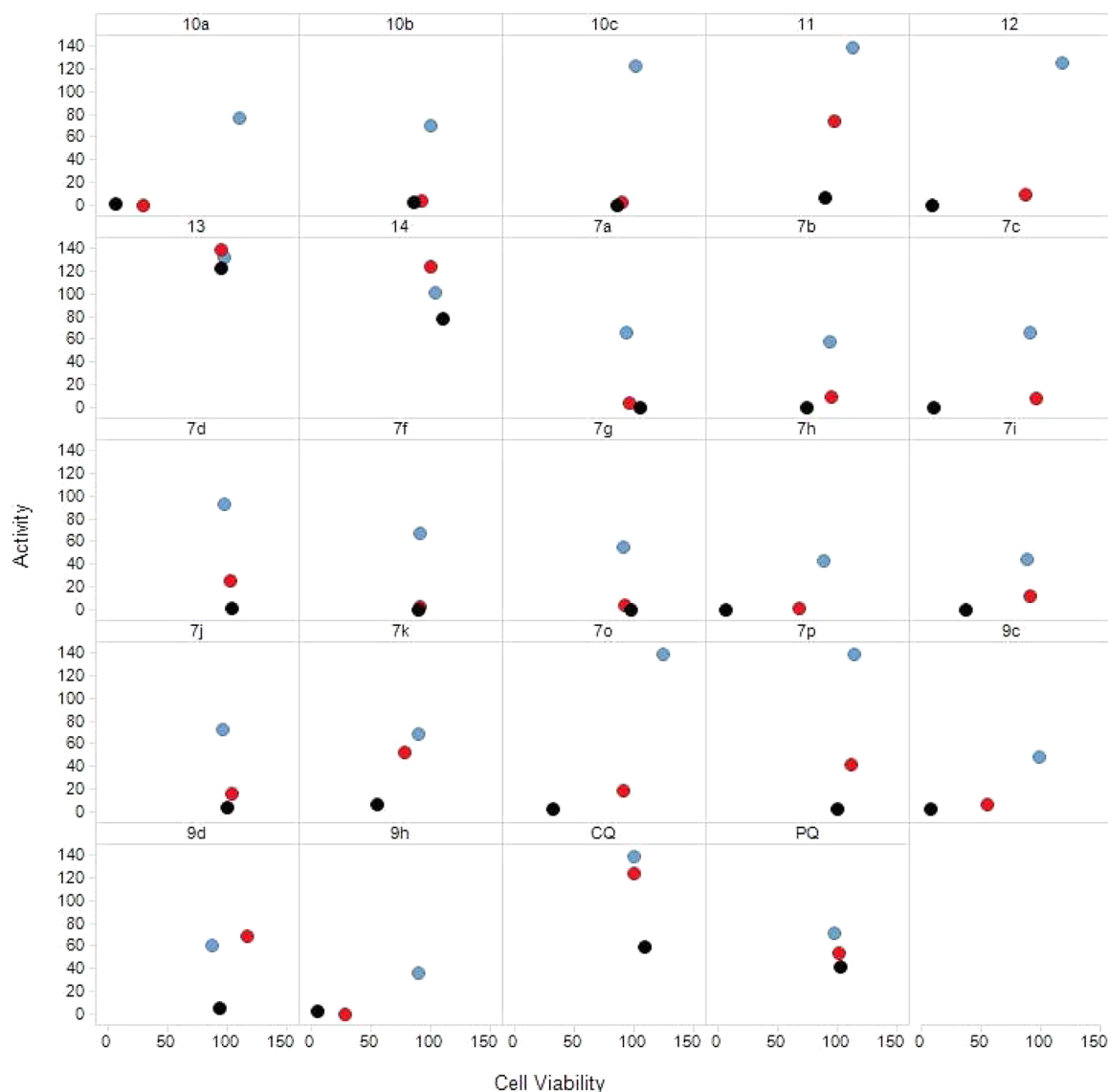
(v) Substitution of the chloroquinoline ring (e.g., 7d,  $IC_{50} \approx 20$  nM) by another heteroaromatic ring, quinolinic (8-amino-6-methoxyquinoline ring in 8d), pyridinic (single heteroaromatic pyridine ring in 13), or nonaromatic (morpholine ring in 14), leads to complete loss of activity.

(vi) Comparison of  $IC_{50}$  values within a given series, e.g., 7 or 9, shows that the cinnamoyl substituent R has some effect on activity but that this effect is not dramatic. The electro-donating/withdrawing character of R seems to have no

significant role in activity, whereas the para position is clearly preferred over the ortho and meta positions, as shown for 7k–l and 9k–l.

(vii) Increase in compound lipophilicity and/or the bulkiness of R within a series seems to enhance in vitro activity. We previously showed that  $IC_{50}$  values for 7 showed good correlation with both  $clogP$  values and Charton parameters.<sup>13</sup> For the new 9 series, we observed a good correlation with Charton parameters but not  $clogP$  values (cf. Table S1 in





**Figure 4.** Activity of HEFLECINs against *P. berghei* liver stages. Anti-infective activity (infection scale, y-axis) and toxicity to hepatoma cells (cell confluency scale, x-axis) are shown. Primaquine (PQ) and chloroquine (CQ) were included for comparison. The black, red, and blue circles represent results for the tested compounds at 10, 5, and 1  $\mu$ M, respectively. Infection loads of Huh7 cells, from a human hepatoma cell line, were determined by bioluminescence measurements of cell lysates 48 h after infection with luciferase-expressing *P. berghei* parasites.<sup>25</sup>

Supporting Information). Series 10 was too small to allow drawing of definitive conclusions in this regard.

(viii) Finally, HEFLECINs bearing the chloroquinoline core were globally more active than CQ, whereas the two most active compounds, 7c and 7h, had activities comparable to that of artemisinin. Therefore, these two compounds, as well as 7d, the most active compound within the series satisfying the leadlikeness,<sup>18</sup> Lipinski<sup>19</sup> and Veber<sup>20</sup> filters, were selected for in vivo studies with *P. berghei*.

**In Vitro Activity against  $\beta$ -Hematin Formation.** The ability of HEFLECINs to inhibit  $\beta$ -hematin formation to hemozoin, the main mechanism attributed to CQ and related antimalarials, was assessed by previously reported methods.<sup>11</sup> This activity was not fully correlated with in vitro antimalarial activities (Table 1), suggesting that HEFLECINs owe their antimalarial properties to mechanisms in addition to inhibition of  $\beta$ -hematin crystallization. Nonetheless, we believe that inhibition of  $\beta$ -hematin formation contributes to activity because (i) variation of  $\beta$ -hematin inhibition potency among

homologues, e.g., 7c, 9c, and 10a, follows the same trend as variation of activities against blood-stage parasites and (ii) the significant decrease in antimalarial activity upon removal of the 7-chlorine substituent (7c vs 12) may be explained by the prior observation that this chlorine atom is essential to ensure effective  $\beta$ -hematin inhibition by CQ and related structures.<sup>21,22</sup>

On the basis of previous and current findings, it is concluded that HEFLECINs do not act against blood-stage parasites via inhibition of the cysteine protease falcipains.<sup>13</sup> Activity may include impairment of the new permeation pathways (NPPs) created by *Plasmodia* in the infected red blood cell (RBC)<sup>13</sup> and inhibition of  $\beta$ -hematin crystallization.

**In Vivo Treatment of *P. berghei* Infected Mice.** For the three most promising HEFLECINs, in vivo efficacy was evaluated using a standard *P. berghei* mouse model. Compounds chosen for in vivo analysis were 7c and 7h because of their potency against cultured blood-stage *P. falciparum* and 7d because of its better druglikeness. Mice were infected intraperitoneally (ip) with *P. berghei* and then

treated twice daily with ip injections of 10, 30, and 100 mg/kg. Chloroquine was used as a positive control at 3, 10, and 30 mg/kg ip twice daily. All mice died because of malaria or toxicity by day 17 at the doses administered. In order to compare compound efficacies, survival over time after the initiation of treatment was compared.

Close inspection of the data leads to the following observations (Figure 3). Compounds **7c** and **7h** were highly toxic at 100 mg/kg, and no mice survived until the last day of treatment. At lower dosages, **7h** extended survival compared to untreated controls by 2–7 days at 30 mg/kg and by 2 days at 10 mg/kg. Compound **7c** did not improve survival compared to untreated controls. Compound **7d** did not appear to be toxic at any tested dosage. It extended survival by 2 days compared to untreated controls at the highest dosage but offered no benefit over untreated controls at the lower dosages.

Studies with the rodent malaria model confirmed that **7d** and **7h** have in vivo antimalarial activity. The lack of in vivo activity of **7c** can be attributed to its high lipophilicity ( $\text{clogP} > 5$ ; see Table S1 in Supporting Information),<sup>19</sup> but compounds **7d** and **7h** are only slightly less lipophilic ( $\text{clogP} \approx 5$ ; see Table S1 in Supporting Information) than **7c**. However, in vivo activities for **7d** and **7h** were lower than might be expected based on their in vitro activities, possibly because of limitations in bioavailability. Additionally, relatively poor performance of the compounds in vivo might be explained by biological differences between *P. berghei* and *P. falciparum* and by the high hydrophobicity of the compounds, which might result in extensive binding to plasma proteins.<sup>23</sup>

**In Vitro Activity against Liver-Stage *P. berghei* Parasites.** Recent findings from our group, demonstrating that the liver-stage activity of PQ is significantly enhanced by conjugation with cinnamic acids, prompted us to investigate whether HEFLECINs would also exhibit liver-stage activity, measured using *P. berghei* as previously reported.<sup>12,17,25</sup> We found that HEFLECINs bearing the chloroquinoline core of CQ (**7**, **9**, **10**) were all active against liver stage parasites. This result was surprising, as CQ is not active against liver-stage malaria.<sup>26</sup> In view of these results, the activities of compounds selected as most promising based on activity and cytotoxicity were compared quantitatively (Figure 4, Table 1). Remarkably, activities were much higher than those observed for either CQ or PQ. Specifically,  $\text{IC}_{50}$  values were 5–15 times lower than those for CQ and 3–7 times lower than those for the reference drug PQ. This is, to our knowledge, the first report of 4-amino-7-chloroquinolinic (CQ-related) structures displaying in vitro activity against liver-stage *Plasmodia* greater than that of PQ. This finding, together with the previous observation that N-cinnamoylation of PQ also enhances its in vitro activity against liver-stage *Plasmodia*,<sup>12</sup> strongly suggests that the N-cinnamoyl moiety is a relevant pharmacophore to boost antiplasmodial activity in both 8- and 4-aminoquinolines. Some SARs could be devised from the liver-stage assays, as follows.

(i) As in blood-stage activity assays, a butyl spacer between the chloroquinoline and the cinnamic cores is generally preferred over propyl and pentyl spacers, in terms of both activity and toxicity, as inferred from comparison of homologues from series **7**, **9**, and **10**.

(ii) Unlike what was observed in blood-stage activity assays, substitution of the cinnamoyl aromatic ring in series **7** led to slightly decreased activity; i.e., the most active compound was **7a**, where  $\text{R} = \text{H}$ .

(iii) The presence of electrodonating R groups in the para position led to a decrease in activity.

(iv) An increase of the number of OMe substituents on the cinnamoyl core from 1 (**7d**) to 2 (**7p**) led to a decrease in toxicity but also in activity.

(v) Removal of the 7-chlorine atom from the chloroquinoline ring led, as observed for blood-stage activity, to a decrease in liver-stage activity.

(vi) Also, as observed against blood-stage *Plasmodia*, replacement of the chloroquinoline ring by pyridine or morpholine led to loss of activity against liver-stage parasites.

As is the case for PQ, the mechanism of HEFLECINs against liver-stage *Plasmodia* remains to be elucidated.<sup>12</sup> Studies are underway to establish the target(s) of HEFLECINs against liver- and blood-stage parasites. The discovery that HEFLECINs bearing the CQ heterocyclic core, unlike their PQ analogues (PRIMACINs<sup>12</sup>), combine significant liver-stage activity with potent blood-stage activity is of great interest. To our knowledge, this is the first example of CQ-related molecules emerging as multistage antimalarial leads.

## ■ CONCLUDING REMARKS

A new family of chloroquine analogues, HEFLECINs, as dual-stage antimalarial leads was found. These compounds display potent in vitro activity against both liver- and blood-stage *Plasmodia*, including chloroquine-resistant blood-stage *P. falciparum* W2 parasites. All of them performed better than chloroquine itself in vitro on both stages, and the best couple of compounds, **7c** and **7h**, were equipotent to artemisinin on blood-stage parasites. Further, all but **11**, **13**, and **14** were better than primaquine on liver-stage parasites. Relevantly, both blood- and liver-stage activities were lost upon replacement of the 4-amino-7-chloroquinoline ring with either a nonaromatic (i.e., morpholine) or an aromatic (i.e., 4-aminopyridine) heterocycle, while replacement with the primaquine's 8-amino-6-methoxyquinoline moiety only eliminated blood-stage activity. This demonstrates that the 4-amino-7-chloroquinoline motif has a critical role for the display of dual-stage antimalarial activity. Two of the most promising compounds were confirmed to be active against the murine model of malarial infection, even though with modest in vivo performances compared to in vitro ones. This is possibly due to bioavailability issues requiring future optimization of the reported antimalarial leads. Studies on the possible mechanism(s) underlying dual-stage activity displayed by the N-cinnamoylated chloroquine analogues are underway. Falcipain inhibition has been ruled out, whereas blocking of  $\beta$ -hematin formation cannot fully account for the activity levels observed. The role of these antimalarial leads as blockers of ion channels specific to infected host cells, like the so-called NPP created in *P. falciparum*-infected RBC, is presently under investigation.

Overall, data show that N-cinnamoylation of chloroquine enhances the parent drug's activity against both blood- and liver-stage *Plasmodia*, which is the first example of chloroquine-derived molecules with multistage antimalarial activity. This finding, along with the recent disclosure of an apparent resensitization of *P. falciparum* to chloroquine in Nigeria,<sup>30</sup> may have relevant impact on the reactivation of chloroquine manufacturers, especially in African countries like Ghana, in which chloroquine production has been halted because of the well-known parasite resistance to this drug.<sup>31</sup>

## ■ EXPERIMENTAL SECTION

**Chemistry. Chemicals and Instrumentation.** All solvents and common chemicals were from Sigma-Aldrich (Spain), whereas Boc-protected amino acids were from NovaBiochem (VWR International, Portugal) and the coupling agent TBTU was from Bachem (Switzerland). NMR spectra were acquired on a Bruker Avance III 400 spectrometer from solutions in either deuterated chloroform or deuterated dimethylsulfoxide (DMSO- $d_6$ ) containing tetramethylsilane as internal reference. Mass spectrometry (MS) analyses were run on a Thermo Finnigan LCQ Deca XP Max LC/MS<sup>n</sup> instrument operating with electrospray ionization and ion-trap (ESI-IT) quadrupole detection. The target compounds were confirmed to have at least 95% purity, based on peak areas obtained through HPLC analyses that were run using the following conditions: 30–100% of B in A (A = H<sub>2</sub>O with 0.05% of trifluoroacetic acid; B = acetonitrile) in 22 min with a flow rate of 1 mL/min on a Merck-Hitachi Lachrom Elite instrument equipped with a diode array detector (DAD) and thermostated (Peltier effect) autosampler, using a Purospher STAR RP-18e column (150 mm × 4.0 mm; particle size, 5 μm).

**Synthesis of Compounds 7–10.** Compounds 7a–l and 8 were prepared by previously described methods, and their analytical and structural data were in perfect agreement with formerly reported data.<sup>12,13</sup> Compounds 7o,p were prepared identically to 7a–l, and their spectroscopic data were compatible with the respective structures, as follows.

**4-[N-(*p*-Dimethylamino)cinnamoylaminobutyl]amino-7-chloroquinoline (7o).** White solid (14 mg, 8%); mp = 176–180 °C;  $R_f$  (DCM/MeOH 8:2) 0.44;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.6 Hz, 2H), 8.32 (d,  $J$  = 8.8 Hz, 1H), 7.95 (m, 1H), 7.79 (d,  $J$  = 2 Hz, 1H), 7.62 (m, 1H), 7.47 (dd,  $J$  = 8.8, 2.4 Hz, 1H), 7.36 (d,  $J$  = 8.8 Hz, 2H), 7.29 (d,  $J$  = 16 Hz, 1H), 6.70 (d,  $J$  = 8.8 Hz, 2H), 6.53 (d,  $J$  = 5.6 Hz, 1H), 6.35 (d,  $J$  = 16 Hz, 1H), 3.32 (m, 2H), 3.22 (m, 2H), 2.94 (s, 6H), 1.68 (m, 2H), 1.57 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.6, 150.9, 150.7, 150.6, 147.6, 138.8, 133.9, 128.7, 126.3, 124.3, 122.3, 117.1, 116.7, 111.9, 98.6, 42.1, 38.1, 26.9, 25.1. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 423.47,  $M^+$  ( $C_{24}H_{27}ClN_4O$ ) requires 422.19. HPLC-DAD:  $t_R$  = 11.7 min, % area = 96.0.

**4-[N-(*m,p*-Dimethoxy)cinnamoylaminopropyl]amino-7-chloroquinoline (7p).** White solid (29 mg, 17%); mp = 168–172 °C;  $R_f$  (DCM/MeOH 8:2) 0.44;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.41 (d,  $J$  = 5.6 Hz, 1H), 8.36 (d,  $J$  = 9.2 Hz, 1H), 8.06 (m, 1H), 7.81 (m, 2H), 7.50 (dd,  $J$  = 9.4 Hz, 2.4 Hz, 1H), 7.34 (d,  $J$  = 16 Hz, 1H), 7.10 (m, 2H), 6.96 (d,  $J$  = 8.4 Hz, 1H), 6.57 (d,  $J$  = 5.6 Hz, 1H), 6.51 (d,  $J$  = 16 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.35 (m, 2H), 3.23 (m, 2H), 1.69 (m, 2H), 1.57 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.2, 151.2, 150.0, 149.8, 148.8, 146.6, 138.4, 134.3, 127.6, 125.5, 124.5, 124.4, 121.2, 119.9, 116.9, 111.7, 109.9, 98.6, 55.4, 55.3, 42.2, 38.2, 26.7, 25.1. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 439.17,  $M^+$  ( $C_{24}H_{26}ClN_3O_3$ ) requires 439.17. HPLC-DAD:  $t_R$  = 12.5 min, % area = 100.

Compounds 9 and 10 were synthesized by methods similar to those previously described for compounds 7.<sup>13</sup> Briefly, 4,7-dichloroquinoline (1 equiv, 1.2 mmol) was reacted with the appropriate diaminoalkane (10 equiv, 12 mmol) at 100 °C for 3 h. The mixture was brought to room temperature and then diluted with dichloromethane (DCM), and the resulting solution was washed with 5% aqueous Na<sub>2</sub>CO<sub>3</sub>. The organic layer was isolated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated to dryness to afford the desired 4-(*N*-aminoalkyl)-amino-7-chloroquinoline (6, Scheme 1). Each compound 6 was then coupled to the respective cinnamic acid in *N,N*-dimethylformamide (DMF), using *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) and *N,N*-diethyl-*N*-isopropylamine (DIEA) (Scheme 1). A solution of cinnamic acid (1.1 equiv, 0.22 mmol), TBTU (1.1 equiv, 0.22 mmol), and 2.2 equiv of DIEA (68 μL) in DMF was stirred at 0 °C for 10 min. A solution of the appropriate compound 6 (1 equiv, 0.2 mmol) in DMF was then added and the reaction allowed to proceed at room temperature for 24 h. The mixture was diluted with DCM, and the resulting solution was washed with 5% aqueous Na<sub>2</sub>CO<sub>3</sub>. The organic layer was separated, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated to dryness, and the crude product was purified by liquid chromatography on silica using EtOAc/

MeOH, 8:2 (v/v), to give the desired compounds 9 and 10. In the particular case of 9e, 4-(*N*-aminopropyl)amino-7-chloroquinoline was first coupled to *p*-(*N*-*tert*-butoxycarbonyl)aminocinnamic acid as above, to yield the *N*-*tert*-butoxycarbonyl (Boc) protected precursor (9e'), followed by removal of Boc through acidolysis with neat trifluoroacetic acid (TFA). Spectral/analytical data on compounds 9 and 10 follow.

**4-[N-(*p*-Cinnamoylaminopropyl)amino-7-chloroquinoline (9a).** White solid (32 mg, 42%); mp 174–192 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.6 Hz, 1H), 8.27 (m, 2H), 7.79 (d,  $J$  = 2.4 Hz, 1H), 7.46 (m, 8H), 6.67 (d,  $J$  = 15.6 Hz, 1H), 6.49 (d,  $J$  = 5.6 Hz, 1H), 3.32 (m, 4H), 1.89 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 172.0, 165.1, 151.9, 150.0, 149.0, 138.5, 134.8, 133.3, 129.3, 128.8, 127.4, 127.4, 124.0, 122.1, 117.4, 98.6, 40.0, 36.6, 27.8. ESI-IT MS:  $m/z$  366.47 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{20}ClN_3O$ ) requires 365.13. HPLC-DAD:  $t_R$  = 13.0 min, % area = 100.

**4-[N-(*p*-Methyl)cinnamoylaminopropyl]amino-7-chloroquinoline (9b).** White solid (53 mg, 68%); mp 204–209 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.6 Hz, 1H), 8.27 (d,  $J$  = 9.2 Hz, 1H), 8.22 (m, 1H), 7.79 (d,  $J$  = 2.4 Hz, 1H), 7.40 (m, 5H), 7.20 (d,  $J$  = 8 Hz, 2H), 6.60 (d,  $J$  = 16 Hz, 1H), 6.48 (d,  $J$  = 5.6 Hz, 1H), 3.32 (m, 4H), 2.30 (s, 3H), 1.87 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.2, 151.8, 149.9, 149.0, 139.1, 138.5, 133.3, 132.1, 129.4, 127.4, 127.4, 124.0, 121.0, 117.4, 98.6, 39.4, 36.6, 27.8, 20.8. ESI-IT MS:  $m/z$  380.4 ( $M + H^+$ )  $M^+$  ( $C_{22}H_{22}ClN_3O$ ) requires 379.15. HPLC-DAD:  $t_R$  = 14.0 min, % area = 100.

**4-[N-(*p*-Isopropyl)cinnamoylaminopropyl]amino-7-chloroquinoline (9c).** White solid (28.7 mg, 33%); mp 173–178 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.2 Hz, 1H), 8.27 (d,  $J$  = 9.2 Hz, 1H), 8.22 (m, 1H), 7.79 (d,  $J$  = 2.4 Hz, 1H), 7.40 (m, 5H), 7.27 (d,  $J$  = 8 Hz, 2H), 6.59 (d,  $J$  = 15.6 Hz, 1H), 6.48 (d,  $J$  = 5.6 Hz, 1H), 3.32 (m, 4H), 2.89 (m, 1H), 1.86 (m, 2H), 1.19 (d,  $J$  = 6.8 Hz, 6H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.2, 151.8, 150.0, 149.9, 149.0, 138.5, 133.3, 132.5, 127.5, 127.5, 126.8, 124.0, 121.1, 117.4, 98.6, 40.0, 36.6, 33.2, 27.8, 23.6. ESI-IT MS:  $m/z$  408.47 ( $M + H^+$ ),  $M^+$  ( $C_{24}H_{26}ClN_3O$ ) requires 407.18. HPLC-DAD:  $t_R$  = 16.0 min, % area = 94.9.

**4-[N-(*p*-Methoxy)cinnamoylaminopropyl]amino-7-chloroquinoline (9d).** White solid (15 mg, 7%); mp 178–180 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.6 Hz, 1H), 8.28 (d,  $J$  = 9.2 Hz, 1H), 8.18 (m, 1H), 7.79 (d,  $J$  = 2.4 Hz, 1H), 7.44 (m, 5H), 6.97 (d,  $J$  = 8.8 Hz, 2H), 6.51 (m, 2H), 3.78 (s, 3H), 3.32 (m, 4H), 1.85 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 172.0, 165.4, 160.2, 151.6, 150.1, 148.7, 138.2, 133.4, 129.0, 127.4, 127.2, 124.0, 119.6, 117.4, 114.2, 98.6, 55.2, 40.0, 36.5, 27.8. ESI-IT MS:  $m/z$  396.53 ( $M + H^+$ ),  $M^+$  ( $C_{22}H_{22}ClN_3O_2$ ) requires 395.14. HPLC-DAD:  $t_R$  = 13.1 min, % area = 98.0.

**4-[N-(*p*-(*N*-*tert*-Butoxycarbonyl)amino)cinnamoylaminopropyl]amino-7-chloroquinoline (9e').** White solid (124.5 mg, 41%); mp 218–220 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.1;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 9.54 (s, 1H), 8.39 (d,  $J$  = 5.6 Hz, 1H), 8.27 (d,  $J$  = 8.8 Hz, 1H), 8.15 (m, 1H), 7.78 (d,  $J$  = 2.4 Hz, 1H), 7.46 (m, 5H), 7.34 (m, 2H), 6.49 (m, 2H), 3.32 (m, 4H), 1.85 (m, 2H), 1.48 (s, 9H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.3, 152.5, 151.8, 149.9, 149.0, 140.7, 138.3, 133.3, 128.5, 128.1, 127.4, 124.0, 123.9, 119.9, 118.0, 98.6, 79.3, 40.0, 36.5, 28.0, 27.8. ESI-IT MS:  $m/z$  481.33 ( $M + H^+$ ),  $M^+$  ( $C_{26}H_{29}ClN_4O_3$ ) requires 480.19. HPLC-DAD:  $t_R$  = 15.0 min, % area = 100.

**4-[N-(*p*-Amino)cinnamoylaminopropyl]amino-7-chloroquinoline (9e).** White solid (28.8 mg, 73%); mp 103–105 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.1;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.2 Hz, 1H), 8.27 (d,  $J$  = 9.2 Hz, 1H), 8.0 (m, 1H), 7.79 (d,  $J$  = 2 Hz, 1H), 7.44 (dd,  $J$  = 8.8 Hz, 2 Hz, 1H), 7.29 (m, 4H), 6.56 (d,  $J$  = 8.4 Hz, 2H), 6.48 (d,  $J$  = 5.6 Hz, 1H), 6.29 (d,  $J$  = 15.6 Hz, 1H), 5.55 (b, 2H), 3.29 (m, 4H), 1.84 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 166.0, 151.8, 150.4, 150.0, 149.0, 139.4, 133.3, 129.0, 127.4, 124.0, 122.1, 117.4, 115.7, 113.6, 98.6, 40.0, 36.5, 27.9. ESI-IT MS:  $m/z$  381.40 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{21}ClN_4O$ ) requires 380.14. HPLC-DAD:  $t_R$  = 8.65 min, % area = 96.0.



**4-[N-(*m*-Fluoro)cinnamoylaminopropyl]amino-7-chloroquinoline (9f).** White solid (36 mg, 45%); mp 185–190 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.2 Hz, 1H), 8.27 (m, 2H), 7.79 (d,  $J$  = 2 Hz, 1H), 7.41 (m, 6H), 7.20 (m, 1H), 6.67 (d,  $J$  = 16 Hz, 1H), 6.48 (d,  $J$  = 5.6 Hz, 1H), 3.33 (m, 4H), 1.87 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 172.7, 164.8, 163.6, 161.2, 151.8, 150.0, 149.0, 137.4, 137.2, 133.3, 130.7, 127.4, 124.0, 123.6, 117.4, 115.9, 113.8, 98.6, 40.0, 36.7, 27.7. ESI-IT MS:  $m/z$  384.40 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClFN_3O$ ) requires 383.12. HPLC-DAD:  $t_R$  = 13.4 min, % area = 100.

**4-[N-(*p*-Fluoro)cinnamoylaminopropyl]amino-7-chloroquinoline (9g).** White solid (27 mg, 34%); mp 187–193 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.2 Hz, 1H), 8.28 (m, 2H), 7.78 (d,  $J$  = 2.4 Hz, 1H), 7.61 (m, 2H), 7.42 (m, 2H), 7.23 (m, 2H), 6.59 (d,  $J$  = 16 Hz, 1H), 6.48 (d,  $J$  = 4.8 Hz, 1H), 3.32 (m, 4H), 1.87 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 172.1, 165.0, 163.8, 161.3, 151.8, 150.0, 149.0, 137.4, 133.3, 129.6, 127.4, 124.0, 122.0, 117.4, 115.9, 98.6, 40.0, 36.6, 27.8. ESI-IT MS:  $m/z$  384.47 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClFN_3O$ ) requires 383.12. HPLC-DAD:  $t_R$  = 13.3 min, % area = 100.

**4-[N-(*p*-Chloro)cinnamoylaminopropyl]amino-7-chloroquinoline (9h).** White solid (39 mg, 46%); mp 191–195 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.6 Hz, 1H), 8.28 (m, 2H), 7.78 (d,  $J$  = 2.4 Hz, 1H), 7.58 (m, 2H), 7.44 (m, 5H), 6.63 (d,  $J$  = 15.6 Hz, 1H), 6.49 (d,  $J$  = 5.6 Hz, 1H), 3.32 (m, 4H), 1.87 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.9, 151.7, 150.1, 148.8, 137.2, 133.8, 133.4, 129.1, 128.9, 127.3, 124.1, 124.0, 122.9, 117.4, 98.6, 40.0, 36.6, 27.8. ESI-IT MS:  $m/z$  400.40 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}Cl_2N_3O$ ) requires 399.09. HPLC-DAD:  $t_R$  = 13.4 min, % area = 95.7.

**4-[N-(*p*-Bromo)cinnamoylaminopropyl]amino-7-chloroquinoline (9i).** White solid (22 mg, 39%); mp 214–219 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (d,  $J$  = 5.2 Hz, 1H), 8.29 (m, 2H), 7.78 (d,  $J$  = 2 Hz, 1H), 7.60 (m, 2H), 7.51 (m, 2H), 7.40 (m, 3H), 6.65 (d,  $J$  = 16 Hz, 1H), 6.48 (d,  $J$  = 5.6 Hz, 1H), 3.32 (m, 4H), 1.86 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.9, 151.8, 150.0, 149.0, 137.3, 134.1, 133.3, 131.8, 129.4, 127.4, 124.0, 123.0, 122.5, 117.4, 98.6, 40.0, 36.6, 27.7. ESI-IT MS:  $m/z$  446.20 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClBrN_3O$ ) requires 443.04. HPLC-DAD:  $t_R$  = 14.7, % area = 100.

**4-[N-(*o*-Nitro)cinnamoylaminopropyl]amino-7-chloroquinoline (9j).** White solid (61.7 mg, 72%); mp 190–195 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (m, 2H), 8.29 (d,  $J$  = 9.2 Hz, 1H), 8.03 (d,  $J$  = 8 Hz, 1H), 7.69 (m, 5H), 7.44 (dd,  $J$  = 9 Hz, 2 Hz, 1H), 7.33 (m, 1H), 6.63 (d,  $J$  = 15.6 Hz, 1H), 6.49 (d,  $J$  = 5.2 Hz, 1H), 3.33 (m, 4H), 1.89 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.2, 151.8, 149.9, 149.0, 148.2, 133.7, 133.5, 133.3, 130.1, 130.0, 128.6, 127.4, 126.8, 124.5, 124.0, 117.4, 98.6, 40.0, 36.7, 27.7. ESI-IT MS:  $m/z$  411.47 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClN_4O_3$ ) requires 410.11. HPLC-DAD:  $t_R$  = 13.0 min, % area = 100.

**4-[N-(*m*-Nitro)cinnamoylaminopropyl]amino-7-chloroquinoline (9k).** Yellow solid (22 mg, 25%); mp 209–214 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (m, 2H), 8.26 (m, 2H), 8.20 (dd,  $J$  = 8 Hz, 1.6 Hz, 1H), 8.01 (d,  $J$  = 7.6 Hz, 1H), 7.77 (d,  $J$  = 2 Hz, 1H), 7.70 (m, 1H), 7.55 (d,  $J$  = 15.6 Hz, 1H), 7.44 (dd,  $J$  = 8.8 Hz, 2.4 Hz, 1H), 7.34 (m, 1H), 6.82 (d,  $J$  = 15.6 Hz, 1H), 6.49 (d,  $J$  = 5.6 Hz, 1H), 3.34 (m, 4H), 1.89 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.5, 151.8, 149.9, 149.0, 148.2, 136.7, 136.2, 133.8, 133.3, 130.4, 127.4, 125.0, 123.8, 123.6, 121.4, 117.4, 98.6, 36.7, 27.7. ESI-IT MS:  $m/z$  411.40 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClN_4O_3$ ) requires 410.11. HPLC-DAD:  $t_R$  = 13.2 min, % area = 95.0.

**4-[N-(*p*-Nitro)cinnamoylaminopropyl]amino-7-chloroquinoline (9l).** Yellow solid (42 mg, 49%); mp 200–205 °C;  $R_f$  (EtOAc/MeOH 8:2) 0.25;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.39 (m, 2H), 8.26 (m, 3H), 7.81 (m, 3H), 7.53 (d,  $J$  = 15.6 Hz, 1H), 7.45 (d,  $J$  = 9 Hz, 2.4 Hz, 1H), 7.35 (m, 1H), 6.81 (d,  $J$  = 16 Hz, 1H), 6.49 (d,  $J$  = 5.6 Hz, 1H), 3.34 (m, 4H), 1.88 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.4, 151.7, 150.0, 149.0, 147.4, 141.5, 136.2, 133.4, 128.5, 127.3, 126.4, 124.0, 124.0, 117.4, 98.6, 36.8, 27.8. ESI-IT MS:  $m/z$  411.40 ( $M + H^+$ ),  $M^+$  ( $C_{21}H_{19}ClN_4O_3$ ) requires 410.11. HPLC-DAD:  $t_R$  = 13.2 min, % area = 96.0.

**4-[N-(*p*-Isopropyl)cinnamoylaminopentyl]amino-7-chloroquinoline (10a).** White solid (190 mg, 50%); mp = 152–155 °C;  $R_f$  (DCM/MeOH 8:2) 0.58;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.38 (d,  $J$  = 5.6 Hz, 1H), 8.28 (d,  $J$  = 9.5 Hz, 1H), 8.07 (m, 1H), 7.77 (d,  $J$  = 2 Hz, 1H), 7.36 (m, 7H), 6.56 (d,  $J$  = 16 Hz, 1H), 6.46 (d,  $J$  = 5.6 Hz, 1H), 3.22 (m, 4H), 2.89 (m, 1H), 1.69 (m, 2H), 1.46 (m, 4H), 1.19 (d,  $J$  = 6.8 Hz, 6H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 164.9, 151.8, 150.0, 149.8, 148.9, 138.2, 133.3, 132.5, 127.4, 127.3, 126.8, 124.0, 123.9, 121.3, 117.3, 98.5, 42.3, 38.5, 33.2, 28.9, 27.4, 24.0, 23.6. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 436.47,  $M^+$  ( $C_{26}H_{30}ClN_3O$ ) requires 435.21. HPLC-DAD:  $t_R$  = 16.3 min, % area = 98.0.

**4-[N-(*p*-Methoxy)cinnamoylaminopentyl]amino-7-chloroquinoline (10b).** White solid (100 mg, 27%); mp = 142.144 °C;  $R_f$  (DCM/MeOH 8:2) 0.58;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.38 (d,  $J$  = 5.6 Hz, 1H), 8.28 (d,  $J$  = 9.2 Hz, 1H), 8.01 (m, 1H), 7.77 (d,  $J$  = 2 Hz, 1H), 7.39 (m, 4H), 6.96 (d,  $J$  = 8.8 Hz, 2H), 6.47 (m, 2H), 3.78 (s, 3H), 3.22 (m, 4H), 1.69 (m, 2H), 1.47 (m, 4H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.1, 160.1, 151.7, 150.0, 148.9, 138.0, 133.3, 128.9, 127.4, 127.3, 124.0, 123.9, 119.8, 117.3, 114.3, 98.5, 55.1, 42.3, 38.4, 28.9, 27.4, 24.0. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 424.40,  $M^+$  ( $C_{24}H_{26}ClN_3O_2$ ) requires 423.17. HPLC-DAD:  $t_R$  = 13.7 min, % area = 100.

**4-[N-(*p*-Chloro)cinnamoylaminopentyl]amino-7-chloroquinoline (10c).** Yellowish solid (65 mg, 17%); mp = 164–168 °C;  $R_f$  (DCM/MeOH 8:2) 0.58;  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.38 (d,  $J$  = 5.2 Hz, 1H), 8.29 (d,  $J$  = 8.8 Hz, 1H), 8.16 (m, 1H), 7.77 (d,  $J$  = 1 Hz, 1H), 7.56 (d,  $J$  = 8.8 Hz, 2H), 7.42 (m, 4H), 7.32 (m, 1H), 6.64 (d,  $J$  = 16 Hz, 1H), 6.44 (d,  $J$  = 5.2 Hz, 1H), 3.22 (m, 4H), 1.68 (m, 2H), 1.51 (m, 2H), 1.41 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 165.1, 160.1, 151.7, 150.0, 148.9, 138.0, 133.3, 128.9, 127.4, 127.3, 124.0, 123.9, 119.8, 117.3, 114.3, 98.5, 55.1, 42.3, 38.4, 28.9, 27.4, 24.0. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 428.53,  $M^+$  ( $C_{23}H_{23}Cl_2N_3O$ ) requires 427.12. HPLC-DAD:  $t_R$  = 14.9, % area = 100.

**Synthesis of Compound 11.** 4-[(7-Chloroquinolin-4-yl)amino]butan-1-ol (11'). An amount of 1 equiv of 4,7-dichloroquinoline (0.5g, 2.5 mmol) was mixed with 4 equiv of 4-aminobutan-1-ol (0.9g, 10 mmol) in a round-bottom flask, and the reaction was allowed to proceed at 100 °C for 4 h. Then DCM (25 mL) was added, followed by addition of 30% aqueous  $Na_2CO_3$  (25 mL), with concomitant precipitation of a light pink solid. The solid was submitted to column chromatography on silica, using DCM/MeOH (8:2 v/v) as eluent, and pure 11' was obtained as a white solid (0.49 g, 78%); mp = 171–174 °C;  $R_f$  = 0.26 (DCM/MeOH 8:2);  $\delta_H$  (400 MHz, DMSO- $d_6$ ) 8.38 (d,  $J$  = 5.2 Hz, 1H), 8.27 (d,  $J$  = 9.2 Hz, 1H), 7.77 (d,  $J$  = 2.4 Hz, 1H), 7.42 (dd,  $J$  = 9.2 Hz, 2.4 Hz, 1H), 7.32 (m, 1H), 6.44 (d,  $J$  = 5.6 Hz, 1H), 4.51 (b, 1H), 3.46 (m, 2H), 3.26 (m, 2H), 1.69 (m, 2H), 1.54 (m, 2H);  $\delta_C$  (100 MHz, DMSO- $d_6$ ) 151.8, 150.0, 149.0, 133.2, 127.4, 124.0, 123.9, 117.4, 98.5, 60.4, 42.2, 30.0, 24.4. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 251.33,  $M^+$  ( $C_{13}H_{15}ClN_2O$ ) requires 250.09.

**4-[N-(*p*-Methoxy)cinnamoyloxypentyl]amino-7-chloroquinoline (11).** An amount of 1 equiv of *p*-methoxycinnamoyl chloride (0.08 g, 0.4 mmol) was reacted with 1 equiv of 11' (0.1 g, 0.4 mmol) in refluxing DCM (2 mL) in the presence of 2 equiv of triethylamine (112  $\mu$ L). The solution turned yellow after 10 min, and the reaction ran for 24 h. The organic layer was washed with 5% aqueous  $Na_2CO_3$  (3  $\times$  2 mL), dried with anhydrous  $Na_2SO_4$ , filtered, and led to dryness on a rotary evaporator. The residue was submitted to column chromatography on silica, using DCM/Me<sub>2</sub>CO (1:1 v/v) as eluent to yield 11 as a white solid (34 mg, 21%); mp = 129–134 °C;  $R_f$  (DCM/Me<sub>2</sub>CO 1:1) 0.26;  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 8.49 (d,  $J$  = 5.2 Hz, 1H), 7.93 (d,  $J$  = 2 Hz, 1H), 7.71 (d,  $J$  = 9.2 Hz, 1H), 7.63 (d,  $J$  = 16 Hz, 1H), 7.43 (d,  $J$  = 8.4 Hz, 2H), 7.31 (d,  $J$  = 8.8 Hz, 1H), 6.88 (d,  $J$  = 8.4 Hz, 2H), 6.39 (d,  $J$  = 5.2 Hz, 1H), 6.28 (d,  $J$  = 16 Hz, 1H), 5.38 (m, 1H), 4.27 (m, 2H), 3.82 (s, 3H), 3.36 (m, 2H), 1.89 (m, 4H);  $\delta_C$  (100 MHz, CDCl<sub>3</sub>) 167.3, 161.4, 151.7, 149.8, 148.8, 144.7, 134.9, 129.7, 128.4, 126.9, 125.3, 121.1, 117.1, 115.1, 114.3, 99.0, 63.7, 55.3, 42.9, 26.5, 25.3. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 411.33,  $M^+$  ( $C_{23}H_{23}ClN_2O_3$ ) requires 410.14. HPLC-DAD:  $t_R$  = 15.4 min, % area = 97.0.

**4-[N-(*p*-Isopropyl)cinnamoylaminopropyl]aminoquinoline (12).** Following the experimental procedure previously described for compounds 9 and 10 and starting from 4-chloroquinoline (0.25 g, 1.5 mmol) instead of 4,7-dichloroquinoline, 12 was obtained as a beige

solid (55 mg, 11%): mp = 73–78 °C;  $R_f$  (DCM/MeOH 8:2) 0.30;  $\delta_H$  (400 MHz,  $CDCl_3$ ) 8.41 (d,  $J$  = 5.6 Hz, 1H), 8.02 (d,  $J$  = 8.4 Hz, 1H), 7.95 (d,  $J$  = 8.4 Hz, 1H), 7.60 (m, 2H), 7.39 (m, 3H), 7.18 (d,  $J$  = 8 Hz, 2H), 6.52 (m, 1H), 6.46 (d,  $J$  = 16 Hz, 1H), 6.34 (d,  $J$  = 5.6 Hz, 1H), 6.15 (b, 1H), 3.46 (m, 2H), 3.35 (m, 2H), 2.89 (m, 1H), 1.74 (m, 4H), 1.22 (d,  $J$  = 6.8 Hz, 6H);  $\delta_C$  (100 MHz,  $CDCl_3$ ) 166.7, 150.9, 149.2, 146.6, 141.0, 132.4, 129.7, 128.0, 127.9, 126.9, 125.0, 120.5, 119.7, 118.5, 98.4, 43.1, 39.1, 34.0, 27.8, 25.5, 23.8. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 388.27,  $M^+$  ( $C_{25}H_{29}N_3O$ ) requires 387.23. HPLC-DAD:  $t_R$  = 15.2 min, % area = 99.0.

**Synthesis of Compound 13. *N*-(Pyridin-4-yl)butane-1,4-diamine (13').** An amount of 1 equiv of 4-chloropyridine (0.5 g, 3.3 mmol) was reacted with 10 equiv of butane-1,4-diamine (2.9 g, 33 mmol) in a round-bottom flask put under reflux for 2 h. After the reaction mixture was cooled to room temperature, an amount of 25 mL of distilled water was added, and the desired product was extracted with DCM (3  $\times$  25 mL). The organic layer was dried over anhydrous  $Na_2SO_4$ , filtered, and led to dryness on a rotary evaporator. This afforded 13' as a yellow oil (0.22 g, 40%). ESI-IT MS:  $m/z$  ( $M + H^+$ ) 166.33,  $M^+$  ( $C_9H_{15}O_3$ ) requires 165.13. Compound 13' was used without further purification in the synthesis of 13.

**4-[*N*-(*p*-Methoxycinnamoyl)butyl]aminopyridine (13).** In a round-bottom flask put at 0 °C, 1.1 equiv of *p*-methoxycinnamic acid (0.29 g, 1.5 mmol) was dissolved in DMF (2.5 mL) and activated by addition of 1.1 equiv of TBTU (0.47 g, 1.5 mmol) and 2 equiv of DIEA (454  $\mu$ L). After 10 min, a solution of 1 equiv of 13' (0.22 g, 1.3 mmol) in DMF (2.5 mL) was added to the previous mixture and the reaction allowed to proceed for 24 h. Water (25 mL) was added to the reaction mixture, and the desired product was extracted with DCM (3  $\times$  25 mL). The organic extract was then washed with 5% aqueous  $Na_2CO_3$  (3  $\times$  25 mL), dried over anhydrous  $Na_2SO_4$ , filtered, and taken to dryness on a rotary evaporator. The residue was submitted to column chromatography on silica, using DCM/MeOH 8:2 (v/v) as eluent, to afford 13 as a white solid (0.13 g, 30%): mp = 132–136 °C;  $R_f$  = 0.13 (DCM/MeOH 8:2);  $\delta_H$  (400 MHz,  $CDCl_3$ ) 8.04 (b, 2H), 7.54 (d,  $J$  = 16 Hz, 1H), 7.33 (d,  $J$  = 8.8 Hz, 2H), 7.28 (m, 1H), 6.77 (d,  $J$  = 8.8 Hz, 2H), 6.36 (m, 3H), 5.14 (m, 1H), 3.73 (s, 3), 3.35 (m, 2H), 3.06 (m, 2H), 1.60 (m, 4H);  $\delta_C$  (100 MHz,  $CDCl_3$ ) 166.8, 160.6, 153.7, 149.0, 140.0, 129.1, 127.4, 118.5, 114.1, 107.4, 55.2, 42.1, 39.1, 27.2, 26.0. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 326.33,  $M^+$  ( $C_{19}H_{23}N_3O_2$ ) requires 325.18. HPLC-DAD:  $t_R$  = 11.6 min, % area = 100.

**Synthesis of Compound 14. *N*-(*N*-Phthaloyl)-aminobutylmorpholine (14').** An amount of 2 equiv of morpholine (0.5g, 5.7 mmol) were reacted with 1 equiv of 1-(*N*-phthaloyl)amino-4-bromobutane (0.8 g, 2.9 mmol) in refluxing DCM for 24 h, after which the reaction mixture was washed three times with 5% aqueous  $Na_2CO_3$ , dried over anhydrous  $Na_2SO_4$ , filtered, and led to dryness in a rotary evaporator. The residue was submitted to column chromatography on silica, using as eluent DCM/MeOH (8:2 v/v), to afford 14' as a white solid (0.53 g, 64%): mp = 61–63 °C;  $R_f$  = 0.67 (DCM/MeOH 8:2);  $\delta_H$  (400 MHz,  $CDCl_3$ ) 7.80 (m, 2H), 7.70 (m, 2H), 3.66 (m, 6H), 2.39 (m, 6H), 1.68 (m, 2H), 1.51 (m, 2H);  $\delta_C$  (100 MHz,  $CDCl_3$ ) 168.4, 133.8, 132.1, 123.1, 66.9, 58.3, 53.6, 37.8, 26.4, 23.8. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 289.40,  $M^+$  ( $C_{16}H_{20}N_2O_3$ ) requires 288.15.

***N*-Aminobutylmorpholine (14').** An amount of 1 equiv of 14' (0.1 g, 0.30 mmol) was reacted with 50% ethanolic hydrazine (0.40 mL) for 2 h at room temperature for removal of the phthaloyl *N*-protecting group. Water was then added to the reaction mixture, and the desired product was extracted with DCM (3  $\times$  25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and led to dryness on the rotary evaporator to afford 14' as a yellowish oil (25 mg, 45%). ESI-IT MS:  $m/z$  ( $M + H^+$ ) 159.27,  $M^+$  ( $C_8H_{18}N_2O$ ) requires 158.14. Compound 14' was used without further purification in the synthesis of 14.

***N*-(*p*-Methoxycinnamoyl)aminobutylmorpholine (14).** An amount of 1 equiv of *N*-aminobutylmorpholine (0.025 g, 0.16 mmol) was immediately used, without further purification, in the coupling with *p*-methoxycinnamic acid (0.032 g, 0.17 mmol) carried out as above-described for 13 but using PyBOP (0.088 g, 0.17 mmol)

instead of TBTU as coupling reagent and DCM as a solvent. This yielded 14 as a white solid (32 mg, 61%): mp = 92–94 °C;  $R_f$  (DCM/MeOH 8:2) 0.52;  $\delta_H$  (400 MHz,  $CDCl_3$ ) 7.59 (d,  $J$  = 15.6 Hz, 1H), 7.40 (d,  $J$  = 8.4 Hz, 2H), 7.20 (d,  $J$  = 8.4 Hz, 2H), 6.34 (m, 2H), 3.72 (m, 4H), 3.38 (m, 2H), 2.90 (m, 1H), 2.44 (m, 4H), 2.37 (m, 2H), 1.60 (m, 4H), 1.23 (d,  $J$  = 6.8 Hz, 6H);  $\delta_C$  (100 MHz,  $CDCl_3$ ) 160.1, 150.7, 140.7, 132.5, 127.7, 126.9, 119.8, 66.9, 58.3, 53.6, 39.5, 34.0, 27.4, 24.0, 23.8. ESI-IT MS:  $m/z$  ( $M + H^+$ ) 331.40,  $M^+$  ( $C_{20}H_{30}N_2O_2$ ) requires 330.23. HPLC-DAD:  $t_R$  = 13.2 min, % area = 100.

**In Vitro Assays. Blood Stage Activity Assays.** The activity of compounds against cultured *P. falciparum* was evaluated as previously reported.<sup>11</sup> Briefly, synchronized ring-stage W2 strain *P. falciparum* parasites were cultured with multiple concentrations of test compounds (added from 1000 $\times$  stocks in DMSO) in RPMI 1640 medium supplemented with 0.5% Albumax (Invitrogen, GIBCO) and 100  $\mu$ M hypoxanthine. After a 48 h incubation, when control cultures contained new rings, parasites were fixed with 1% formaldehyde in PBS, pH 7.4, for 48 h at room temperature and then labeled with 1 nM YOYO-1 (Molecular Probes) in 0.1% Triton X-100 and 100 mM ammonium chloride in PBS. Parasitemias were determined from dot plots (forward scatter vs fluorescence) acquired on a FACSort flow cytometer using CELLQUEST software (Becton Dickinson). IC<sub>50</sub> values for growth inhibition were determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration. In each case, goodness of curve fit was documented by  $R^2 > 0.95$ .

**Inhibition of  $\beta$ -Hematin.** The  $\beta$ -hematin inhibition assay was performed as previously described.<sup>27,28</sup> Different concentrations (0.1–1 mM) of test compounds dissolved in DMSO were added in triplicate to 50  $\mu$ L of hemin chloride dissolved in DMSO (5.2 mg/mL). Negative controls were water and DMSO.  $\beta$ -Hematin formation was initiated by the addition of acetate buffer 0.2 M (100  $\mu$ L, pH 4.4). Plates were incubated at 37 °C for 48 h, and they were then centrifuged at 3000 rpm for 15 min (Sigma 3–30 K). After the supernatant was discarded, the pellet was washed four times with DMSO (200  $\mu$ L) and finally dissolved in 0.2 M aqueous NaOH (200  $\mu$ L). The solubilized aggregates were further diluted 1:6 with 0.1 M aqueous NaOH, and absorbances were recorded at 405 nm (Biotek Powerwave XS with software Gen5 1.07).

**Liver-Stage Infection Assays.** Inhibition of liver-stage infection by test compounds was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase expressing *P. berghei* line, *PbGFP-Luc<sub>con</sub>*, as previously described.<sup>29</sup> Huh-7 cells, from a human hepatoma cell line, were cultured in 1640 RPMI medium supplemented with 10% v/v fetal calf serum, 1% v/v nonessential amino acids, 1% v/v penicillin/streptomycin, 1% v/v glutamine, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7, and maintained at 37 °C with 5% CO<sub>2</sub>. For infection assays, Huh-7 cells (1.2  $\times$  10<sup>4</sup> per well) were seeded in 96-well plates the day before drug treatment and infection. Medium in the cells was replaced by medium containing the appropriate concentration of each compound approximately 1 h prior to infection with sporozoites freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes. Sporozoite addition was followed by centrifugation at 1700g for 5 min. At 24 h after infection, medium was replaced by fresh medium containing the appropriate concentration of each compound. Inhibition of parasite development was measured 48 h after infection. The effect of the compounds on the viability of Huh-7 cells was assessed by the AlamarBlue assay (Invitrogen, U.K.), using the manufacturer's protocol.

**In Vivo Assays. Evaluation of the in Vivo Antimalarial Effects of HFELECIINS.** Female Swiss Webster mice ( $n$  = 5) were infected with 10<sup>6</sup> *P. berghei*-infected erythrocytes by ip injection. Two hours later the treatment was initiated with ip injections of 10, 30, and 100 mg/kg compounds 7c, 7d, and 7h twice daily for 4 days. Chloroquine was used as a positive control at 3, 10, and 30 mg/kg ip twice daily. For all murine malaria experiments, mice were evaluated daily for toxicity and for parasitemia by evaluation of Giemsa-stained blood smears. Animals



were sacrificed when significant toxicity was identified or when parasitemias topped 50%.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Structures of heterocycle cinnamic acid conjugates and Table S1 of the physicochemical descriptors for N-cinnamoylated CQ analogues 7 and 9. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

<sup>13</sup>C NMR, carbon-13 nuclear magnetic resonance; <sup>1</sup>H NMR, proton nuclear magnetic resonance; ACT, artemisinin-based combination therapy; CQ, chloroquine; DCM, dichloromethane; DIEA, N-ethyl-N,N-diisopropylamine; DMF, N,N-dimethylformamide; DMSO-d<sub>6</sub>, deuterated dimethylsulfoxide; ESI-IT, electrospray ionization and ion-trap quadrupole detection; HEFLECIN, heterocycle-cinnamic conjugate; HPLC-DAD, high performance liquid chromatography with diode array detection; MS, mass spectrometry; NPP, new permeation pathway; PQ, primaquine; PRIMACIN, primaquine-cinnamic acid conjugate; RBC, red blood cell; SAR, structure-activity relationship; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

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