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Reinvestigating Old Pharmacophores: Are 4-Aminoquinolines and Tetraoxanes Potential Two-Stage Antimalarials?

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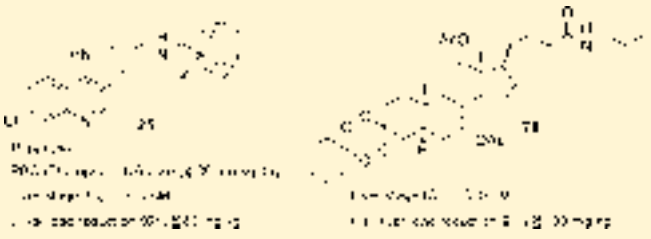
Supporting Information

ABSTRACT: The syntheses and antiplasmodial activities of various substituted aminoquinolines coupled to an adamantane carrier are described. The compounds exhibited pronounced in vitro and in vivo activity against *Plasmodium berghei* in the Thompson test. Tethering a fluorine atom to the aminoquinoline C(3) position afforded fluoroaminoquinolines that act as intrahepatocytic parasite inhibitors, with compound **25** having an IC_{50} = 0.31 μM and reducing the liver load in mice by up to 92% at 80 mg/kg dose. Screening our peroxides as inhibitors of liver stage infection revealed that the tetraoxane pharmacophore itself is also an excellent liver stage *P. berghei* inhibitor (**78**: IC_{50} = 0.33 μM). Up to 91% reduction of the parasite liver load in mice was achieved at 100 mg/kg. Examination of tetraoxane **78** against the transgenic 3D7 strain expressing luciferase under a gametocyte-specific promoter revealed its activity against stage IV–V *Plasmodium falciparum* gametocytes (IC_{50} = 1.16 ± 0.37 μM). To the best of our knowledge, compounds **25** and **78** are the first examples of either an 4-aminoquinoline or a tetraoxane liver stage inhibitors.



INTRODUCTION

Malaria is a widespread parasitic disease affecting mainly the population of Sub-Saharan Africa, South-East Asia, and South America. With its heavy burden of ca. 207 million cases in 2013 and a death toll of ca. 627000, malaria is one of the major public health problems worldwide (WHO Malaria Report 2014).[1] In addition, with as much as ca. 10% of the global population being transiently ill and temporarily unable to work, malaria poses an immense economic burden to malaria-endemic regions. Fortunately, over the last 15 years, the death toll and the number of cases decreased steadily by the combined action of the widespread use of insecticide-treated bed nets, the implementation of artemisinin-based combination therapies (ACTs),[2] and appropriate diagnosis.

In particular, ACTs, in which a fast parasitemia clearing peroxide molecule is combined with a slow-acting antimalarial pharmacophore, an aminoquinoline, or the like, proved to be a very effective therapeutic approach. Thanks to the public and private contributions and the efforts of Medicine for Malaria Venture (MMV), new combinations (i.e., DHA–piperaquine; pyronaridine–artesunate) or formulations (i.e., artemether–lumefantrine dispersible for children) have been approved and distributed.[3]

Nevertheless, drug resistance already pushed aside many good and low-toxic antimalarial drugs, most relevantly, chloroquine. A few years ago, the first signs of resistance of *Plasmodium falciparum* (*Pf*) to artemisinin in Western Cambodia were reported as unusual prolonged parasite clearance phenotype following treatment with an ACT.[4,5] Artemisinin resistance may presently be spreading throughout South-East Asia.[6] Multiple point mutations in the propeller domains of the *Pf* kelch gene on chromosome 13 (K13), have been associated with the resistance phenotype in vivo and represent a molecular marker for tracking the spread of artemisinin resistance.[7]

In this context, to counter the problems outlined above, new, cheap, and effective antiplasmodial drugs are needed. The life

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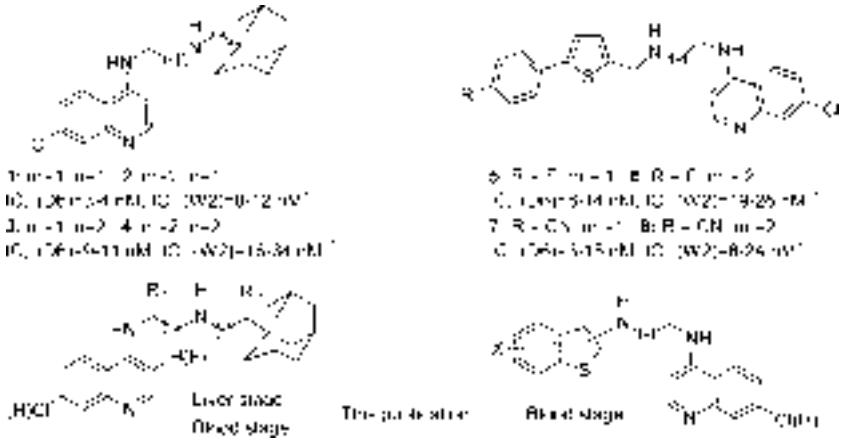
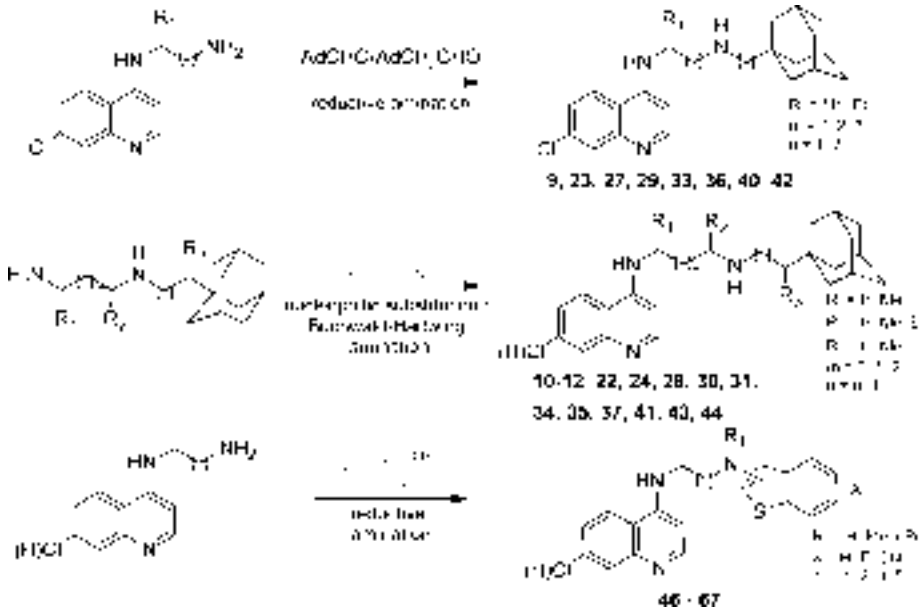


Figure 1. Recently developed aminoquinoline antimalarials.

Scheme 1. Amination Affords Antiplasmodials 9−12, 22−24, 27−31, 33−37, 40−44, and 46−67



cycle of *Plasmodium* parasites includes several stages in both its mammalian and its invertebrate hosts. Malaria infection in mammals starts with the bite of an infected female *Anopheles* mosquito, followed by migration of *Plasmodium* sporozoites to the liver, where they infect and develop inside hepatocytes. Hepatic infection is asymptomatic and malaria symptoms do not arise until hepatic merozoites are released into the bloodstream and infect erythrocytes. Therefore, *Plasmodium* liver stages appear to be promising targets for new antimalarial drugs aiming at prophylactic intervention.[8] In the blood, the asexual cyclic replication of the parasites causes the symptoms of malaria and leads to the differentiation of the parasite's sexual forms, the gametocytes, which are then taken up by a mosquito, where the sexual phase of the parasite's life cycle takes place. The discovery and development of antimalarials that act through several, if not all, stages of the parasite life cycle would greatly enhance their effectiveness, thus allowing much cheaper and considerably more effective regimens. This is also the most recent indication of MMV for new antimalarials.[3]

The activity of several chemotypes that target multiple stages of the parasite's life cycle was reported in the past few years: (+)-SJ733,[9] imidazopyrazines,[10] quinolone-3-diarylethers,[11] flavones,[12] *N*-cinnamoylated chloroquine analogues,[13] quinolin-4(1*H*)-imines,[14] 3,5-bis(benzylidene)-4-piperidones,[15] imidazopyrazines,[16] primaquine−chloroquine hybrid,[17] ketotifen, norketotifen,[18] decoquinate,[19] and thienopyrimidinones.[20] All these multiple stage inhibitors are active against both liver and blood *Plasmodium* asexual stages. Remarkably, imidazopyrazines,[10,16] quinolone-3-diarylethers,[11] primaquine−chloroquine hybrid,[17] and decoquinate[19] are active against blood sexual stages too. In vivo liver-stage (LS) activity was confirmed for imidazopyrazines,[10,16] quinolone-3-diarylethers,[11] primaquine−chloroquine hybrid,[17] ketotifen, norketotifen,[18] decoquinate,[19] and fluoroquinoline DDD107498.[21]

Peroxide-containing hybrid compounds, in which the LS drug primaquine was bound to an artemisinin or tetraoxane moiety, were synthesized as potential new antimalarial drugs, active against both the liver and the erythrocytic asexual stages of the parasite. In fact, both peroxide classes possess excellent intraerythrocytic activity. Several primaquine−artemisinin (PQ-ART) hybrids,[22] tetraoxane−pyrimidine nitrile hybrids,[23] and tetraoxane−, and artemisinin−primaquine hybrids[24] were reported. The PQ-ART and tetraoxane−primaquine amalgams showed very interesting LS activity in vitro. However, the

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observed LS activity could be primarily ascribed to the partner employed in the hybrids molecules (based on the relative LS inactivity of the respective partner peroxide entities—tetraoxanes and ART[23,25,26]). The in vivo LS activity was confirmed for primaquine—ART hybrids.[22]

Recently, it was observed that reducing drug (chloroquine) pressure results in re-emergence of chloroquine-sensitive *P. falciparum* malaria.[27] Although it is very unlikely that any aminoquinoline would be used in monotherapy regimens, new aminoquinolines were developed as promising antimalarials and as possible partners in combination treatments.[28−36] 7-Chloro-4-aminoquinolines (7-ACQ) are considered remarkably active against asexual blood stages but not against LS parasites.[25] It is therefore not surprising that only one successful effort toward designing a LS-active 7-ACQ has been reported.[17] Lödige et al. showed that a PQ—CQ hybrid is efficient against asexual blood stages of CQ-susceptible and CQ-resistant *P. falciparum* strains 3D7, Dd2, and K1, and, importantly, against in vitro LS of *P. berghei*.[17] In addition, they showed that the PQ—CQ hybrid molecule is able to appreciably decrease the parasite liver load when dosed at 3× 90 mg/kg.

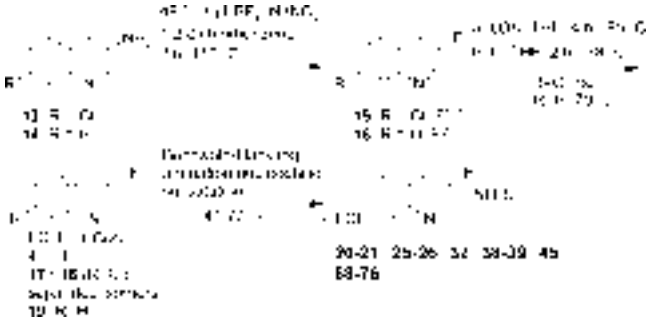
Our own efforts in the field of 7-ACQ antimalarials resulted in promising compounds with adamantane[37] and thiophene-based carriers (Figure 1).[38]

Here, we report on a new generation of aminoquinolines with adamantane carriers and benzothiophene terminal amine substitutions. We show that compounds of the new series have improved blood stage antiplasmodial activity in comparison to the initial series. In addition, the first LS activity of an 4-aminoquinoline is reported for the first time, followed by first-in-class LS activity of a tetraoxane antimalarial.

RESULTS

Chemistry. Here, we present the synthesis of three types of aminoquinoline antiplasmodials: (a) adamantane derivatives, (b) benzothiophene derivatives, and (c) 3-fluoro-4-aminoquinolines, which include chloroquine and AQ-13 fluoro derivatives (Scheme 1, Scheme 2). The key reaction for the preparation of adamantane and benzothiophene *P. falciparum* inhibitors is the coupling of prepared fragments by reductive, or Buchwald—Hartwig amination as outlined in Scheme 1. Full details on the syntheses of these compounds and their respective precursors are given as part of Supporting Information.

Scheme 2. Synthesis of 7-Chloro-3-fluoro-4-aminoquinolines[a]



[a]R_1: (*N*-alkyl-2-(adamant-1-yl)alkanamine); 5-(diethylamino)pentan-2-yl, 3-(diethylamino)propan-1-yl.

The synthesis of the 3-fluoro-substituted aminoquinoline core was initiated with the introduction of fluorine at the C(3) position via corresponding diazonium salt (not isolated, Balz—Schiemann reaction[39]) in 70% yield, starting with known 3-aminochloroquinolines **13** and **14** Scheme 2. The introduction of iodine in position C(4) was accomplished using low temperature generation of intermediate lithio species followed by low temperature iodination thereof,[40] affording the iodine regioisomers (**17** and **18**, R = Cl) in (4:1) ratio. It is interesting to note that when R = H, the lithiation/iodination sequence afforded only 3-fluoro-4-iodoaminoquinoline (**19**) in 70% yield. The synthesis of the aminoquinoline part was concluded by Buchwald—Hartwig or nucleophilic amination (41—77%, Scheme 2). The yields of amination reactions at C(2) and C(4) varied; the cross-coupling reaction proceeded smoothly except on two occasions when possibly steric congestion of fluorine and a short spacer lowered the reaction yields (compounds **20** and **68** in Supporting Information). All compounds screened for antiplasmodial activity were fully characterized and were found to be >95% pure (HPLC).

Plasmodium Asexual Blood Stages. In Vitro Antiplasmodial Activity. Synthesized compounds were tested in vitro for their antiplasmodial activity against three *P. falciparum* strains: D6 (CQ susceptible (CQS) strain), W2 (CQ resistant (CQR) strain), and TM91C235 (Thailand, a multidrug-resistant (MDR) strain), by measuring the inhibition of incorporation of radiolabeled hypoxanthine by drug-treated parasites, as previously reported.[41] CQ, mefloquine (MFQ), and artemisinin (ART) were used as positive controls. The antiplasmodial activity of all compounds tested is given in Table S1 (Supporting Information). Twenty-one of these compounds were more potent than CQ and MFQ (IC_{50}) against the CQS strain D6, and 23 were more active against MDR C235 strain than MFQ. Of 49 compounds more active than CQ against the CQR strain W2, 26 were ≥10-fold more active against the given strain. In addition, three compounds, **9**, **33**, and **34**, were more active than ART against all three *P. falciparum* strains tested.

The in vitro activity data of the most potent compounds with the adamantane carrier is selected to Table 1. Detailed analysis revealed several interesting SAR issues within the series differing in linker length:

In general, a series with shortest linker (m = 1, Scheme 1) was more active than the ones with m = 2,3 against all three *P. falciparum* strains (compounds **9**, **10**, **33**, **34**). All four compounds had excellent selectivity indices against HepG2 and RAW264.7 cells ($SI_{HepG2/D6}$ = 4188; $SI_{RAW\ 264.72/D6}$ = 1886—2901), indicating that they could constitute good candidates for in vivo evaluation. The introduction of methyl groups in the α-positions to nitrogens (in an attempt to subdue possible metabolic hydroxylation of the respective α-carbons) afforded remarkably more active antiplasmodials, e.g., **9**, **10**, **33**, and **34** vs **1** (for chemistry and activity details of **1** see ref 37); however, the screening in human and mouse liver microsomal preparations indicated that only **33** and **34** are acceptably metabolically stable (MLM). Compounds **33** and **34** (m = 1, n = 2) appeared to be 2.5—6 times more stable than those with shorter linkers, m = 1, n = 1 (Table 1). In addition, the position of the methyl group in N—C(α)—C(α′)—N—C(α″) linker also had a favorable effect on activity: antiplasmodials **9**, **33** (CH_3—C(α)) vs **10**, **34** (CH_3—C(α′), Table 1) and **11** (CH_3—C(α″), Supporting Information, Table S1).

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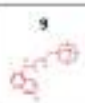
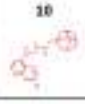
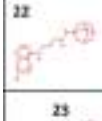
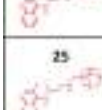
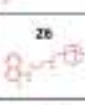
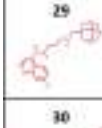
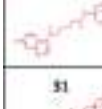
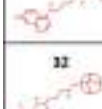
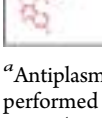
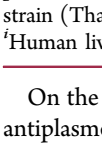
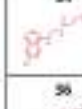
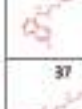
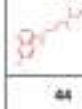
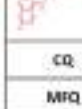
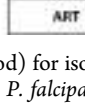
Table 1. In Vitro Antiplasmodial Activity

Compound	MW	In vitro antimalarial activity[a,b] (*P. falciparum*, IC_{50}, nM)			Toxicity HepG2[f] (RAW 264.7)[g] IC_{50}, nM	MLM[h] (min)	HLM[i] (min)	SI HepG2 (RAW) /D6
		D6[c]	C235[d]	W2[e]				
9 	383.97	1	1	3	(2901)	10	11	(2901)
10 	383.97	2	9	10	(3771)	10	13	(1886)
22 	398.00	4	15	22	(3161)	11	16	790
23 	398.00	10	10	10	3045	60	60	305
24 	363.54	17	135	185	2317	60	60	136
25 	415.99	135	197	238	3867	21 (79[j])	13 (78[j])	29
26 	381.53	60	259	157				3
29 	412.02	21	23	22	24271	57	49	1156
30 	412.02	7	19	25	3080			441
31 	412.02	51	165	282	1612			32
32 	430.01	198	342	488	3549			18
33 	398.00	1	1	4	(2910)	60	57	(2910)
34 	398.00	6	7	7	25127	60	27	4188
36 	412.02	13	13	13	24271	60	60	1867
37 	377.57	7	102	126	1590			227
38 	430.01	143	171	203	3212			22
39 	395.56	154	540	394				3
42 	426.05	7	14	26	13273	60	60	1896
43 	426.05	9	47	81	2598			289
44 	391.99	12	280	393	1593			133
45 	444.04	288	590	547	2608			9
CQ	319.87	12	139	456				
MFQ	378.31	16	36	5				
ART	282.33	9	13	7				

[a]Antiplasmodial IC_{50} values (nM) ([^{3}H]hypoxanthine incorporation method) for isolates and clones of *P. falciparum*. [b]All in vitro experiments were performed as technical triplicates with R2 within 0.96−1. [c]CQ susceptible *P. falciparum* African D6 clone. [d]*P. falciparum* multidrug resistant C235 strain (Thailand). [e]CQ resistant *P. falciparum* Indochina W2 clone. [f]Hepatocellular carcinoma. [g]Rat macrophage cell line. [h]Mouse liver microsomes. [i]Human liver microsomes. [j]Mouse hepatocytes. [k]Human hepatocytes.

On the basis of the above findings we turned our attention to antiplasmodials with methyls at C(α) position in the series with extended linkers:

The examination of the series with m = 2, n = 1, and n = 2 (Scheme 1, Ad derivatives) revealed that antiplasmodials with a 7-chloro-4-aminoquinoline moiety (**22**, **23**, **24**, **36**, and **37**) had acceptable SI (136−1,867), but we found low SI values for fluorine derivatives **25** and **38** (29, 22, respectively) as well as considerable in vivo toxicity issues for compound **39** (vide infra). In this series, good activity against CQS D6, CQR W2, and MDR C235 strains was afforded by 7-chloro derivatives **22**, **23**, and **36** (4−22 nM, Table 1). To directly compare the influence of chlorine at C(7), des-chloroaminoquinolines **24** and **37** were prepared along with their respective fluoro derivatives **26** and **39**. We found that omitting the Cl−C(7) substituent did not affect the in vitro activity of **24** and **37** against the CQS D6 strain (IC_{50} = 17 and 7 nM, respectively, Table 1; cf. compounds **35** and **44**, Supporting Information, Table S1). However, the activity against the CQR W2 and MDR C235 strains was significantly impaired; all des-chloro compounds were 8−86-fold less active than their 7-chloro-4-aminoquinoline analogues against the MDR C235 strain and 10−24-fold less active against the CQR W2 strain (**23** vs **24**; **36** vs **37**; **33** vs **35**; **42** vs **44**, Table 1 and Supporting Information, Table S1). The introduction of a fluorine atom in position C(3) failed to improve the in vitro activity. All F−C(3) derivatives

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Table 2. In Vivo Antimalarial Activity (Thompson Test)[a,b]

compd	mg/kg/day	parasitemia	mice dead/day died	mice alive on day 31/total	mean survival time (MST, day)
1	40	D6: negative, clearance. Two mice positive at D31	3/15−24	2/5	>21.4
34	80	D3: 3 mice 0.4−0.5%; D6: 3 mice negative; D10: 2 mice negative, 1 mouse 0.2%; D14%: 3 mice 1.6−3.6%	1/14, 1/15, 1/17	0/3	15.3
33[c]	160	D6: mice negative; D24: 3 mice 2.7−9.6%; D28:1 mouse 67.4%. D31: 2 mice negative	2/24, 1/28	2/5	27.6
23	160	D3: 5 mice 0.7−1.2%; D6: 5 mice negative; D17: 5 mice negative; D31: 5 mice negative		5/5	>31
	80	D3: 5 mice 0.4−0.5%; D6: 5 mice negative; D17: 5 mice negative; D31: 5 mice negative		5/5	>31
	40	D3: 5 mice 0.3−2.4%; D6: 5 mice negative; D10: 5 mice negative; D14: 5 mice 0.2−1.2%; D17: 2 mice 2.1−4.6%	1/16, 2/17, 2/18	0/5	17.2
	20	D3: 4 mice 0.5−3.5%; D6: 4 mice negative; D10: 4 mice 0.2−0.4%; D14: 2 mice 1−3.9%; D17: 1 mouse 3.5%	2/14, 1/15, 1/18	0/4	15.2
	10	D3: 5 mice 0.4−1.6%; D6: 5 mice 0.18−0.5%; D10: 5 mice 1.6−8.9%	1/11, 3/13, 1/15	0/5	13
24	80	D3: 5 mice 0.2−1%; D6: 5 mice negative; D10: 3 mice negative, 2 mice 0.2−0.9%; D14: 5 mice 1.4−2.5%	2/14, 3/16	0/5	15.2
25	160	D3: 5 mice 0.3−0.5%; D6: 1 mouse ex, 4 mice negative; D17: 4 mice negative; D31: 4 mice negative	1/7	4/5	26.2
	80	D3: 6 mice 0.3−1%; D6: 6 mice negative; D17: 6 mice negative; D31: 6 mice negative		6/6	>31
	40	D3: 5 mice 0.5−3%; D6: 5 mice 1−4.7%; D10: 5 mice 3.1−16.3%; D14 :3 mice 5.6−23%; D21: 2 mice 37.5−62.4%	2/12, 1/21, 1/23, 1/24	0/5	18.4
26	80	D3: 5 mice 0.4−1.4%; D6: 5 mice 1.3−26.8%; D10: 3 mice 3.2−25%; D14→D21: 1 mouse 7.7%→35%	2/8, 1/12, 1/13, 1/23	0/5	12.8
42	160	D6: 5 mice negative; D14: 4 mice negative, 1 mouse 0.3%; D17: 3 mice recrudescence, 1 mouse negative	1/17, 1/19, 1/21, 1/24, 1/26	0/5	21.4
	40	D6−D14: 0.4−52.3%	1/12, 4/14	0/5	13.6
74	160	D3: 5 mice 0.5−1.5%; D6: 5 mice negative; D10: 2 mice negative, 3 mice 0.1−0.4%; D14: 5 mice 0.16−3.4%; D17: 2 mice 6.9−10%	1/15, 1/16, 1/17, 2/18	0/5	16.8
73	160	D3: 5 mice 0.3−1.3%; D6: 5 mice negative; D10: 5 mice 0.2−0.4%; D14: 5 mice 3−7%	3/14, 1/15, 1/16	0/5	14.6
75	160	D3: 6 mice 0.1−1.5%; D6: 6 mice 0.2−2.6%; D10: 5 mice 1.7−4.9%; D14: 4 mice 9.6−23.5%; D21: 4 mice 52−58%; D28: 1 mouse 60%	1/9, 1/12, 2/22, 1/27, 1/29	0/6	20.2
58	80	D3: 4 mice 0.2−0.7%; D6: 4 mice 0.2−0.3%; D10: 4 mice 0.4−0.6%; D14: 4 mice 1.3−2.7%	4/15	0/4	14
59	160	D3: 5 mice 0.9−2.3%; D6: 5 mice negative; D10: 5 mice negative; D14: 5 mice negative; D17: 5 mice 0.3−4.4%; D21: 4 mice 2.3−3.8%; D31: 3 mice positive	1/18, 1/21	3/5	26.4
63	160	D3: 4 mice 0.4−0.9%; D6: 4 mice negative; D10: 3 mice negative, 1 mouse 0.2%; D14: 4 mice 0.2−1.2%; D17: 4 mice 0.5−1.7%; D21: 4 mice 1−37%; D31: 3 mice positive	1/27	3/4	30
	80	D3: 5 mice 0.1−1.7%; 5 mice negative; D10: 5 mice 0.2−0.4%; D14: 5 mice 0.6−3.5%; D17: 5 mice 1.2−6.5%; D21: 5 mice 2.4−15.2%; D31: 1 mouse positive	1/27, 3/31	1/5	30.2
CQ	160	D3: 5 mice 0.4−0.9%; D6−D31: 5 mice negative		5/5	> 31
	80	D3: 5 mice 0.5−0.9%; D6: 5 mice negative; D10: 5 mice negative; D14: 3 mice negative, 2 mice 0.2%; D17: 3 mice negative, 1 mouse 4%; D21: 3 mice negative; D31: 3 mice negative	1/17, 1/18	3/5	25.6
infected controls	0	all mice died on day 6−8			

[a]Groups of five *P. berghei* (ANKA strain) infected mice were treated with aminoquinolines suspended in 0.5% hydrohyethylcellulose−0.1% Tween 80 administered po once per day on days on 3−5 postinfection. Mice alive on day 31 with no parasites in a blood film were considered cured. [b]All compounds were tested in separate experiments (mouse groups) for toxicity. None exerted any toxic effect when dosed 3 × 160 mg/kg/day. [c]Body temperatures were recorded to predict death according to IACUC protocols and parasitemia was captured prior to euthanizing the animal. The animal euthanasia point was 35.5 °C.

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with the adamantane carrier, **25**, **38**, **26**, **39**, **32**, and **45** (Table 1) and **20** and **21** (Supporting Information, Table S1), were significantly less active than their C(3)-H isosteres. On the nM scale, they could be considered virtually inactive. The lack of in vitro activity of C(3) fluorine derivatives was extended to CQ derivative **74**, which is ca. 34-fold less active than CQ against the CQS D6 strain (Supporting Information, Table S1).

The thiophene moiety was investigated on several occasions as an aromatic contributor to the aminoquinoline pharmacophore.[34,38] As part of this report, we analyzed the effect of the benzothiophene contributor to the aminoquinoline moiety on antiplasmodial activity (Supporting Information, Table S1, Table 2). Two series of this class were prepared: C(2)- and C(3)-substituted benzothiophenes. As a general remark, we note that benzothiophenes did not improve the in vitro activity of aminoquinolines and that the whole series was less potent than the 7-chloro-4-aminoquinoline−thiophene−benzene combination.[38] C(2)-substituted benzothiophenes were found less active in vitro than their C(3) isomers, with only **52** being as active as CQ and MFQ against *P. falciparum* strains D6 and C235, respectively (Supporting Information, Table S1). Of 12 C(3) substituted benzothiophenes, five antiplasmodials were more active than CQ against the CQS strain D6 and six were more active than MFQ against the MDR strain C235. Compounds without a chlorine atom at the quinoline C(7) position (**54**, **62**, **66**) were much less active than the whole benzothiophene series against all three *P. falciparum* strains. The introduction of fluorine and cyano group at positions C(5) and C(6) (benzothiophene numbering) did not appreciably influence the benzothiophene−aminoquinoline hybrids' in vitro activity.

In Vivo Blood-Stage Efficacy Studies. The compounds presented in Table 2 were examined for their in vivo efficacy in a mouse model. A modified Thompson test was employed to determine the blood schizonticidal efficacy of 14 compounds. In addition, in a separate host toxicity study, groups of five healthy mice were dosed with 160 mg/kg/day ×3 days of **34**, **33**, **23**, **24**, **25**, **42**, **74**, **73**, **75**, **58**, **59**, and **63**, without any overt clinical manifestations of toxicity. This was monitored by observations of individual mouse behavior and appearance two times a day for 31 days. Compound **58** was toxic at 160 mg/kg/day and was therefore tested at 80 mg/kg/day at which concentration it did not exibit any signs of toxicity.

Compounds were administered po at given doses on days 3, 4, and 5 after infection using the Thompson test in C57Bl6 female mice infected with 1× 10^6 *P. berghei* parasites.[42] The compounds with an ethylene linker (**1**, **34**, **33**) cleared the parasites on D6. However, recrudescence occurred on days 10−14 and mice succumbed to malaria later on. Only at the higher dose of 160 mg/kg/day did the compound **33** cure two mice, while the minimal active dose (MAD) for **1**, and **34** was estimated at 40 and 80 mg/kg/day, respectively. Next, we tested the series of compounds with a propylene linker: **23**, **24**, **25**, and **26**. The 7-chloro-4-aminoquinoline isosteres **23** and **25** were tested in a dose-dependent manner, and a minimal curative dose (MCD) of 80 mg/kg/day for both compounds was estimated. Although none of the two compounds showed curative effect at the lower doses employed, a small difference was noticed: aminoquinoline **23** was more potent than **25**, exhibiting MAD even at 10 mg/kg/day, and clearance with recrudescence at 40 and 20 mg/kg/day, while **25** was ineffective at 20 and 10 mg/kg/day (not shown). The analogues of **23** and **25**, isosteres **24** and **26**, were selected for examining the in vivo 4-aminoquinoline activity dependence on the chlorine substituent at C(7), at 80 mg/kg/day. As shown in Table 2, none of the compounds cured infection. However, isostere **24** (H-C(3)) was more active than **26**, providing clearance with recrudescence at the administered concentration.

Next, we examined CQ-, and AQ-13-fluoro derivatives **74** and **73**, respectively, at the nontoxic dose of 160 mg/kg/day. Both compounds led to clearance with recrudescence, with no cure even at such a high dose.

Three benzothiophenes were examined for their efficacy at the selected doses (Table 2), and because 5-fluorobenzothiophene **58** was toxic at the higher dose, it was tested at 80 mg/kg/day with no cure or survival. Its F-C(6) isomer **59** was examined at the 160 mg/kg/day dose and exhibited clearance with recrudescence and survival of 3/5 animals, with MST > 26 days (D31: 3 mice positive). The higher homologue of **58**, compound **63**, also provided clearance with recrudescence with an MST of 30 days and survival of 3/4 animals. However, a dramatic difference emerged between the two homologues at 80 mg/kg/day. While **58** provided no clearance, homologue **63** led to clearances with recrudescence, 1/5 survival, and twice the MST of **58**.

Inhibitory Activity of *Plasmodium* Liver Stages. *In Vitro LS Activity.* In an attempt to develop multistage antimalarials, the possible LS activity of our aminoquinoline blood stage antiplasmodials was investigated. The evaluation of the LS activity of our steroidal and nonsteroidal tetraoxanes was also performed by measuring the luminescence intensity in human hepatoma cell line (Huh7) cells infected with a firefly luciferase-expressing *P. berghei* line, as previously described.[43]

Initially, we evaluated the aminoquinoline blood-stage antiplasmodials with an adamantane carrier **23**, **36**, **25**, **38**, and **69** for LS activity using primaquine as a positive control (Table 3). The percentage of Huh7 cell confluency was used as a measure of relative cell toxicity (Figure 2).

The in vitro results presented in Table 3 indicate that 7-ACQ-Ad antiplasmodials **23**, **25**, **36**, **38**, and **69** all exhibit relatively high potency against the liver forms of the parasite. LS inhibitory activity was assessed at seven concentrations (10−0.1 μM), and several compounds showed a dose-dependent behavior (Figure 2). Table 3 and Figure 2 show that at 5 μM concentration, homologues **23** and **36** appear to be potent inhibitors of LS infection (0.1%, 8%, respectively). However, their activity dropped at lower concentrations, yielding IC_{50} values (1−2.5 μM, 2.5−5 μM), comparable to that of AQ-13 and CQ.[25] We then explored the effect of the F-C(3) aminoquinoline substituent on LS inhibitory activity by assaying another homologue pair of fluoro isosteres **25** (←**23**) and **38** (←**36**). It was found that fluorine at C(3) significantly enhanced the activity of **25** in comparison to **23** (IC_{50}= 0.31 μM vs IC_{50} = 1−2.5 μM, respectively), and of **38** in comparison to **36** (IC_{50} = 1−2.5 μM vs IC_{50} = 2.5−5 μM, respectively). Significant in vitro inhibitory activity (38%) of **25** was observed even at 125 nM concentration.To the best of our knowledge, the 3-fluoro compound **25** is the first 4-aminoquinoline that exerts evident and significant in vitro activity against *Plasmodium* LS (Figure 2). The effect of the fluorine on *Plasmodium* LS stage is additionally indicated by enhanced activity of 2-aminoquinoline **69** (a regioisomer of **25**, LS infection of **69** at 5 μm ∼2%, IC_{50} = 1−5 μM), and the activities of CQ fluoro derivative **74** and its 2-aminoquinoline

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Table 3. Liver Stage Activity of 4-Aminoquinoline and Tetraoxane Antiplasmodials

compd	% LS infection (5 μM)[a]	LS IC_{50} (μM)[b]	LS in vivo (mg/kg/day)[c]
23	0.1	1−2.5	
25	13 (2 μM)	0.31 ± 0.14	16 (50); 8 (80)
69	∼2	1−5	
36	8	2.5−5	
38	3	1−2.5	
74	∼35	1−5	
76	∼40	1−5	
77	31	<1	
78	0.55	0.33 ± 0.05	40 (50); 9 (100)
PQ	62 (10 μM)		
CQ		ca. 9[25]	
ART	>75[23]	ca. 12[25]	
ATQ		22 nM[25]	

[a]10000 Huh7 cells/well seeded 24 h before infection; 10000 PbA-LuciGFPcon spz/well. Confluency measured by a fluorimetric assay. Infection was measured by a bioluminescence assay. [b]All dose responses employed at least five dosages. [c]Measurement of liver load by qRT-PCR or by luminescence measurement. Mean of two independent experiments. All mice were infected by iv injection of 10000 *P. berghei* sporozoites. Control groups were treated by oral gavage with vehicle (equivalent % of DMSO, in sunflower oil). Experimental groups were treated by oral gavage administration of **25** and **78** at the given doses.

analogue **76** (both IC_{50}= 1−5 μM), Table 1, Figure 2. For comparison, LS activity of CQ is ∼9 μM.[25]

Tetraoxanes were coupled to pyrimidine-nitriles[23] and primaquine,[24] and the respective hybrids were found to be active against LS *Plasmodium* infection in vitro. During our work on steroidal and nonsteroidal tetraoxane molecules, we exposed their unexpected stability to various reaction conditions (e.g., stability to LAH, pH1.6), and during in vitro metabolism studies we noticed that tetraoxane moiety was resistant to metabolic transformations.[44−46] Therefore, we decided to evaluate the activity of these compounds during the LS of parasite development. Tetraoxanes evaluated here have proven to be excellent intraerythrocytic antimalarials without any observed toxicity (Supporting Information, Figure S1, Table S2).[44−49]

The structures of the two most LS active steroidal tetraoxanes are given in Figure 3, and their respective activities are presented in Table 3.

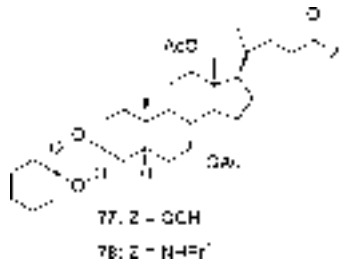

Figure 3. Liver stage active tetraoxanes.

The two tetraoxanes differing only in their side chain exhibited pronounced inhibition potencies with *n*-Pr amide **78** being ca. 3-fold more active than the respective methyl ester **77** (IC_{50} = 0.33 μM vs IC_{50} = <1 μM, respectively). While methyl ester **77** was as active as artemether, the in vitro potency of *n*-Pr amide **78** was comparable to the only LS active peroxide, artemisone (IC_{50} ∼ 0.04 μM[25]). Although the activity of other examined tetraoxanes was lower than that of **77** and **78** (Supporting Information, Table S2), the overall results confirm that the tetraoxane moiety itself is probably capable of killing hepatic parasites without the aid of the 8-aminoquinoline moiety, which would lead to primaquine-like in vitro LS inhibition.

Tetraoxane **78** was also found to be moderately active when tested in vitro against stage IV−V *P. falciparum* gametocytes of the 3D7elo1-pfs16-CBG99 transgenic strain.[50] Compound **78** inhibited gametocytes viability with IC_{50} = 1.16 ± 0.37 μM (mean of three different experiments in duplicate). Control drugs employed were DHA (IC_{50}= 0.44 ± 0.29 μM) and epoxomicin (IC_{50} = 11.8 ± 4.2 nM).

*In Vivo LS Activity of 7-ACQ and Tetraoxane Molecules **25** and **78**.* To obtain a better insight into LS activity of our most active aminoquinoline **25** and tetraoxane **78**, we examined their

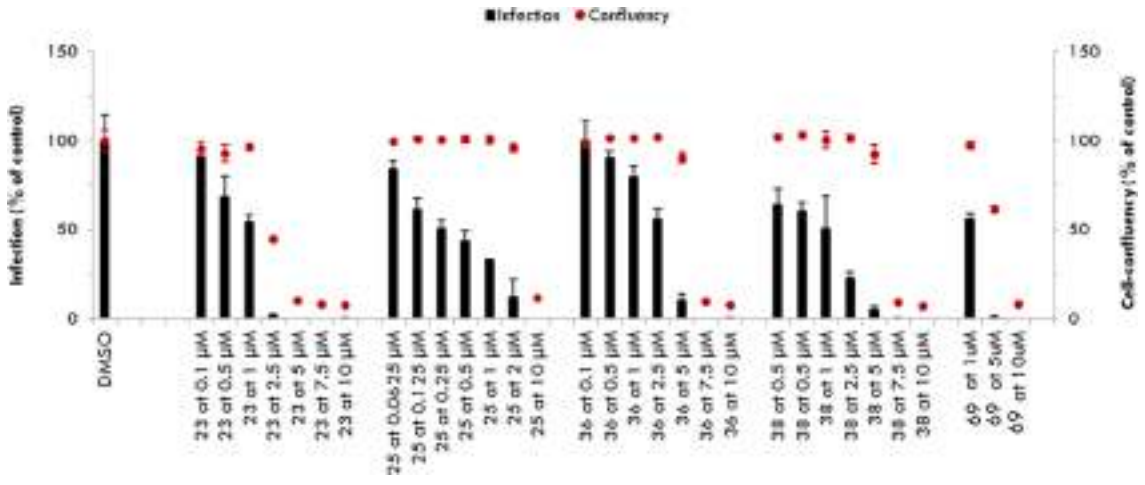

Figure 2. Activity of compounds **23**, **25**, **36**, **38**, and **69** against *P. berghei* liver stages. Anti-*P. berghei* activity (infection scale in relative luminescence units (RLU), bars) and toxicity to Huh7 cells (cell confluency scale in relative fluorescence units (RFU), circles) are shown at several concentrations. The infection load of human hepatocellular carcinoma (Huh-7) cells was determined by bioluminescence measurements of cell lysates 48 h after infection with luciferase-expressing *P. berghei* parasites.

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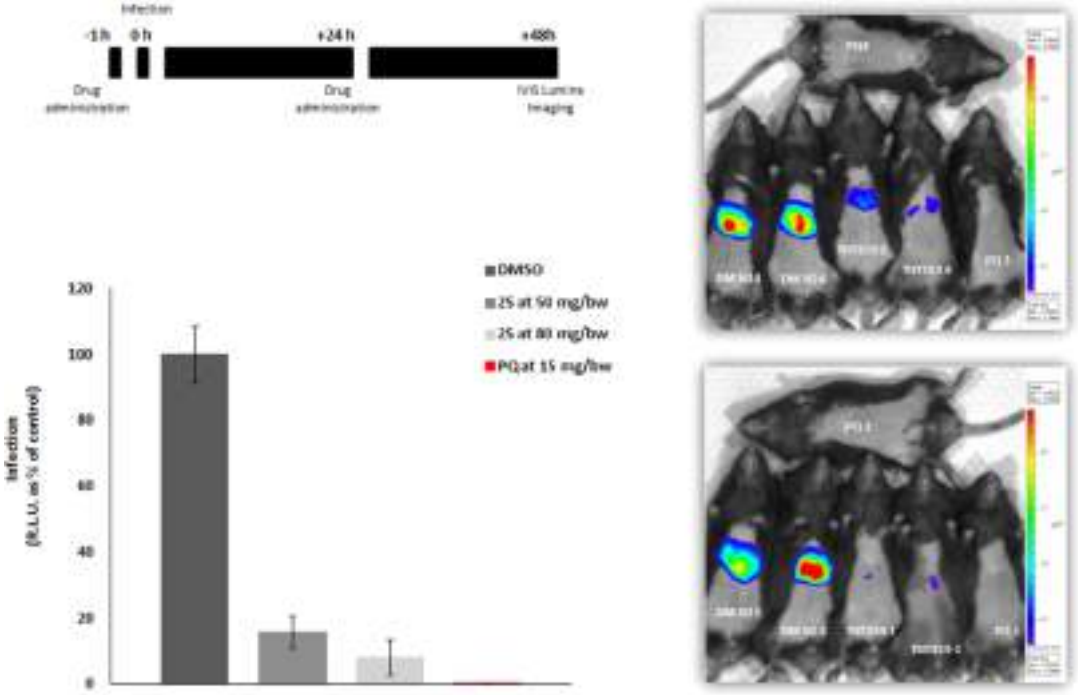

Figure 4. Luminescence-based measurement of liver parasite loads after oral administration of compound **25** measured 48 h after infection and plotted as percentage of the infection levels in control mice.

in vivo activity against *P. berghei* LS. Thus, two groups of five C57BL/6J mice were treated orally with **25** (DMSO + sunflower oil) at 50 and 80 mg/kg doses during three consecutive days (D − 1, D0, D + 1, two independent experiments each). Mice were infected with luciferase-expressing *P. berghei* sporozoites on D0, followed by assessment of liver parasite load 48 h after infection by luminescence, and of the appearance of parasites in the blood, disease symptoms, and survival. Our experiments employing 50 and 80 mg/kg of the compound showed an 84% and 92% decrease of the *P. berghei* liver load relative to controls, respectively (Figure 4).

In a similar experiment tetraoxane **78** administered at 50 and 100 mg/kg reduced the *P. berghei* liver load 60% and 91%, respectively (Figure 5, Table 3).

Discussion. The effect of numerous substituents at C(5), C(6), and C(7) of aminoquinoline antiplasmodials is well explored.[51] However, the antimalarial data on C(3) substituted aminoquinolines are scarce,[52−54] and the introduction of several alkyl/aryl groups at C(3) as the logical consequence of the slightly improved activity of purified sontochin over that of chloroquine revealed that 4-aminoquinolines with C(3) alkyl/aryl substituents ("pharmachins")[55] are generally more active against CQR strains than CQ in vitro. We sought to investigate the effect of a fluorine substituent at aminoquinoline position C(3) on β-hematin formation and on in vitro and in vivo blood stage activity; in addition, we investigated the activity of our inhibitors against *P. berghei* LS.

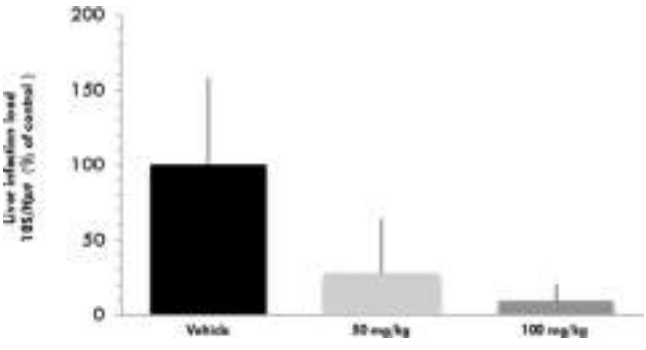

Figure 5. Parasite liver load in C57BL/6 mice measured 48 h after infection by qRT-PCR of *P. berghei* 18S rRNA, normalized to hypoxanthine−guanine phosphoribosyltransferase (*Hprt*) and plotted as percentage of the infection levels in control mice.

As part of our wider investigation of 4-aminoquinoline−adamantane combination as pharmacophore−carrier symbiosis,[37] aminoquinoline **42** was first tested in vivo due to its favorable microsomal metabolic stability and excellent SI data (Table 1, Supporting Information, Table S1). Its rather high logPow (QPlogPow 5.67,[56] experimental 5.87) was assisted by favorably predicted apparent MDCK cell permeability QPPMDCK = 1.29 × 10^{-5} cm/s, rather low predicted QPlogHERG toxicity IC_{50} = −5.076 (8.40 μM), and its MTD was estimated at >160 mg/kg (no host toxicity in mice up to 160 mg/kg/day ×3 days). At 160 mg/kg/day, **42** showed excellent suppression activity on D6-D14 (Table 2). However, the suppression activity was maintained only with 1/5 mice on D17 and recrudescence occurred in 3/5 cases. MST was estimated at 21.4 days without any survival. Screening the compounds containing the ethylene bridge (m = 1, Figure 1) with lower $logP_{ow}$ **1**, **33**, and **34** (Table 2) showed that only **33** cured 2/5 mice at 160 mg/kg/day. Next, we focused on compounds with a propylene linker (m = 2, Figure 1) and with F-C(3) substituents. As shown in Scheme 2 all F-C(3) derivatives with an adamantane carrier, **25**, **38**, **26**, **39**, **32**, **45** (Table 1), and **20**, **21** (Supporting Information, Table S1), were significantly less active in vitro than their H-C(3) isosteres. Homologues **23** and **36** possessed excellent potency across all three *P. falciparum* strains, displayed excellent microsomal metabolic stability (Table 1), and were therefore

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tested in vivo; in addition, their fluorine isosteres **25** and **38** were also chosen for examination in a *P. berghei* challenge assay despite the lower in vitro activity and low microsomal stability of **25**. The respective des-chloro derivatives **24**, **26**, **37**, and **39** were also prepared in order to explore the influence of Cl−C(7) substituent at the aminoquinoline moiety in a *P. berghei* challenge test. First, the compounds were tested for toxicity in mice at doses of 160 mg/kg/day ×3 days. To our surprise, compounds **36**, **37**, **38**, and **39** (all m = 2, n = 2; Scheme 1) were all found to be toxic regardless of their respective substitution pattern at C(3) and C(7), which is in sharp contrast to compounds **42** (m = 3, n = 2), **23**, **24**, **25**, and **26** (m = 2, n = 1) and other compounds listed in Table 2. It is interesting to note that in vivo toxicity results could not be predicted based on in vitro toxicity parameters (cf. RAW 264.7, HepG2 activity, Table 1).

As indicated above, isosteres **23** and **25** cured all mice from blood-stage malaria in the Thompson test when dosed with 160 or 80 mg/kg/day, respectively. The difference between the two became evident at lower doses with isostere **23** providing clearance on D6 even at 20 mg/kg/day. Fluoro derivative **25**, which demonstrated cures of blood stage malaria, was found very active against LS *P. berghei* infection. The activity difference (and lack of mouse toxicity) between the two isosteres warranted further investigation of their MOA. Thus, elementary information about possible mechanism of action was obtained by submitting compounds **23** and **25**, **24** and **26**, fluoro AQ-13 derivative **73**, F-CQ derivative **74**, and its isomer **76**, and CQ as standard, to a β-hematin inhibitory activity assay (BHIA).[57] The assay screens the interference of potential antimalarials with the heme detoxification process employed by *P. falciparum*. The results obtained indicated that the introduction of fluorine at the C(3) position of 4-aminoquinolines greatly enhances the inhibition of β-hematin formation: **23** (1.84) vs **25** (0.61), **24** (1.22) vs **26** (0.61), **CQ** (1.23) vs **74** (1.07), all IC_{50} values, cf. Supporting Information, Table S1. The influence of the F-C(3) substituent is consistent: it significantly enhances the BHIA and indicates that such compounds would strongly interfere with heme detoxification process within FV. However, this observation did not completely match with the compounds' antimalarial activity: all tested fluoro derivatives exhibited slightly lower in vivo activity with respect to their H isosteres (Table 2). Additional information on the influence of amine regiochemistry on BHIA is obtained from the activity of **76**, the 2-aminoalkyl isomer of F-CQ **74**. The lower activity of **76** as compared to **74** (IC_{50} = 2.43 vs IC_{50} = 1.07, respectively) indicates that the interference of alkylaminoquinolines with the hematin formation probably depends on the position of the alkylamino side chain as well.

The introduction of the F-C(3) substituent had a profound effect on the respective dipoles (Supporting Information, Table S1), e.g., **23** (6.04) vs **25** (3.10), log *P*, **23** (3.85) vs **25** (3.25), and p*K*a values. Experimentally determined p*K*a values given in Supporting Information, Table S1 clearly indicate that all examined fluoro derivatives are monoprotonated at physiological pH and to a great extent in FV. Therefore, it appears that while 7-ACQ **23** interacts with hematin as diprotonated species, its fluoro isostere **25** is expected to be mostly monoprotonated in FV, as well as within the liver hepatocytes, where it exerts considerable inhibition of intrahepatocytic parasites (for distribution diagrams see Supporting Information). Because the fluoro derivative **25** showed remarkable in vivo activity both against LS and in asexual blood stages, we checked this compound for hepatocyte metabolic stability followed by metabolite profiling. The intrinsic clearance of 8.9 and 8.7 μL/min/10^6 cells (human (h), mouse (m), respectively) was estimated, therefore, significant stability in hepatocytes resulted: $t_{1/2}$ = 78 min, 79 min (h, m), contrary to the reported poor metabolic stability when incubated with hepatic microsomes (Table 1). The amount of remaining parent compound **25** at 120 min is 32% (h) and 37% (m). Metabolite identification exposed the monohydroxylation product as the main one, and the product of dihydroxylation as minor, after 60 min: **25** : **25+16** : **25+32** = 35:26:1 (h); **25** : **25+16** : **25+32** = 33:26:1 (m). No phase II products were detected. Further metabolite analysis, isolation of the main metabolite, and its testing will be carried out.

Arrhythmia can be induced by blockage of hERG channels by certain antimalarial agents such as lumefantrine[58] and quinidine.[59] Both **23** and **25** were submitted to hERG test, and IC_{50} > 5 μM was estimated for both. At 5 μM concentration (highest concentration tested), low inhibition was found: **23**, 11.7%; **25**, 8.54% (positive control quinidine IC_{50} = 2.67 μM). The Ames test used to assess the mutagenic potential of **23** afforded positive results: antiplasmodial **23** was negative at 500 μg/mL against *Salmonella typhimurium* strains TA98 and TA100 (+S9, −S9).

Current data on isosteres **23** and **25** indicate that both 4-aminoquinolines with adamantane carrier are nontoxic and nonmutagenic antiplasmodials, and they are relatively metabolically stable in microsomal preparations (**23**) and in hepatocyte preparations (**25**). Both compounds are efficient in vivo against *P. berghei* asexual RBC stages and a possible indication on their MOA was obtained from BHIA experiments, which showed that all fluoro 7-ACQ-Ad derivatives were ca. 2-fold more active than their hydrogen isosteres. Benzothiophene antiplasmodials were also found to be more active than CQ. This might suggest that our compounds exert their activity on asexual RBC stages by interfering with the *P. falciparum* detoxification process within the FV. Having in mind the pronounced two-stage activity of **25** and the lack of host toxicity in mice for >31 days (indicating the nontoxicity of monohydroxylation metabolite too), a strategy for the elucidation of LS MOA will be developed.

In vitro LS activities of tetraoxanes with cholic acid carrier appear to be higher than the simple cyclohexylidene analogues and their IC_{50} fall within ca. 1−5 μM (Table S2). The highest activity was observed for *N*-propyl amide **78** (IC_{50}= 0.33 μM), and the proof of concept was obtained after in vivo experiments with **78** reducing the *P. berghei* liver load by 91% at 100 mg/kg. Their mechanism of action is not clear at present; however, because the only pharmacophore present is the tetraoxane moiety, we speculate that peroxide-generated radicals could be involved in parasite elimination.

Asexual *P. falciparum* blood stage infection is followed by the development of sexual parasite forms, gametocytes, which are transmitted to mosquitoes to continue their development. Each round of schizogony is followed by differentiation of merozoites (ca. 1%) into the male and female gametocytes through stages I−V. Tetraoxane **78** was found to moderately inhibit the viability of *P. falciparum* late gametocytes (stage IV−V) with IC_{50} = 1.16 ± 0.37 μM, which is only 3.5-fold higher than the IC_{50} against LS. Our results indicate that tetraoxane **78** constitutes a good starting material for upgrading this pharmacophore against LS and late stage gametocytes, in addition to its established asexual erythrocytic stage activity.

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CONCLUSION

The syntheses and antiplasmodial activities of variously substituted 4-aminoquinolines coupled to adamantane carrier were described. The compounds exhibited pronounced in vitro and in vivo activity against *P. berghei* in the Thompson test. The series with the benzothiophene carrier showed lower activity against D6, C235, and W2 *P. falciparum* strains in comparison to the adamantane series and did not afford any cure in vivo. Tethering fluorine atom to C(3) position of 4-aminoquinoline yielded fluoroaminoquinolines that constitute intrahepatocytic parasite inhibitors, with compound **25** having IC_{50} = 0.33 μM. Compound **25** afforded up to 92% reduction of the in vivo *P. berghei* liver load at 80 mg/kg dose. Testing our peroxide antimalarials as inhibitors of LS infection revealed that the tetraoxane pharmacophore itself is also a powerful LS *P. berghei* parasite inhibitor (**78**: IC_{50} = 0.33 μM). A 91% reduction of the parasite liver load in mice was achieved at 100 mg/kg. To the best of our knowledge, compounds **25** and **78** are the first examples of an 4-aminoquinoline and a tetraoxane liver stage inhibitor, respectively. Neither of the compounds was toxic in vivo, and both possessed good metabolic stability. Their mode of action in the LS is currently unknown. However, in the case of **78**, it could be anticipated that it is likely that peroxide-generated radicals are involved in parasite elimination because the only pharmacophore present is a tetraoxacyclohexane moiety. Compound **25** and its hepatocyte monohydroxylated metabolite are not toxic, as confirmed by challenging the host, **25** is not mutagenic, and has a good hERG profile. The results warrant focusing on the MOA of aminoquinoline **25** and its monohydroxylated metabolite, as well as of tetraoxane **78** against *Plasmodium* LS.

Finally, the compounds exhibiting their action through two (**25**) or more (**78**) parasite stages have good potential to be developed into efficient inhibitors to add to already existing antimalarial drugs.[3] The results presented here open the door to new LS-active chemotypes.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Boetius PMHK apparatus and were not corrected. IR spectra were taken on a Thermo-Scientific Nicolet 6700 FT-IR diamond crystal. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively) and a Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) in the indicated solvent (vide infra) using TMS as the internal standard. Chemical shifts are expressed in ppm (δ) and coupling constants (J) in Hz. ESI–MS (HRMS) spectra of the synthesized compounds were acquired on a Agilent Technologies 1200 series instrument equipped with Zorbax Eclipse Plus C18 (100 mm × 2.1 mm i.d. 1.8 μm) column and DAD detector (190–450 nm) in combination with a 6210 time-of-flight LC/MS instrument in positive and negative ion modes. The samples were dissolved in MeOH (HPLC grade). The selected values were as follows: capillary voltage 4 kV; gas temperature 350 °C; drying gas 12 L min^{-1}; nebulizer pressure 45 psig; fragmentator voltage: 70 V. Mass spectral analyses were performed using electrospray ionization in positive ion mode on a Surveyor separations module coupled to a ThermoFinnigan TSQ_AM triple quadrupole mass spectrometer. Compounds were analyzed for purity using an Agilent 1200 HPLC system equipped with Quat pump (G1311B), injector (G1329B) 1260 ALS, TCC 1260 (G1316A), detector 1260 DAD VL+ (G1315C), and Waters 1525 HPLC dual pump system equipped with an Alltech Select degasser system, and a dual k 2487 UV–Vis detector. LKB 5060–006 micro plate reader (Vienna, Austria) was used for BHIA test. Potentiometric titrations were performed using CRISON pH-Buret 24 2S equipped with CRISON 50 29 microcombined pH electrode (Crison Instruments, SA, Spain). The electrode was calibrated by means of a strong acid–strong base titration in 0.1 M NaCl in MeOH:H_2O (1:1, v:v), using GLEE, GLass Electrode Evaluation software;[60] standard potential E^0 = 397.9 ± 0.2 mV, slope 59.2 ± 0.1 mV, and pK_W 13.84 ± 0.01 were obtained as mean values of four titrations. All compounds were >95% pure. For details, see Supporting Information.

*N*1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine (**AQ2**), *N*1-(7-chloroquinolin-4-yl)propane-1,3-diamine (**AQ3**), *N*1-(7-chloroquinolin-4-yl)butane-1,4-diamine (**AQ4**), *N*1-(7-chloroquinolin-4-yl)-ethane-1,6-hexane (**AQ6**), *N*-(quinolin-4-yl)propane-1,3-diamine (**AQ7**), and *N*-(quinolin-4-yl)butane-1,4-diamine (**AQ8**) were prepared according to known procedures.[61]

General Procedures for Amination. *Compounds **9, 27, 33, and 40.*** To a solution of an amine in CH_2Cl_2 were added the appropriate aldehide (1 equiv) and $NaBH(OAc)_3$ (2 equiv). After stirring the reaction mixture at rt for 24 h, aqueous NaOH was added. The organic layer was separated, and the aqueous layer was washed with CH_2Cl_2. The organic layer was dried over anhyd Na_2SO_4, and the solvent was evaporated under reduced pressure.

*Compounds **23, 29, 36, 42, 46, 49, and 52–67.*** Amine (1.25–1.5 equiv) and appropriate aldehyde (1 equiv) were dissolved in MeOH/CH_2Cl_2 mixture (v:v; 2:1), anhydrous AcOH (1.25–1.5 equiv) was added, and the mixture was stirred under Ar at rt for 3 h. Then, $NaBH_4$ (6 equiv) was added, and stirring was continued for another 18 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The organic layer was washed with 2 M NH_4OH, water, and brine, and dried over Na_2SO_4. Finally, the solvent was evaporated under reduced pressure.

*Compounds **10, 11, 20–22, 28, 30–31, 34–35, 41, 43, and 68.*** A mixture of diamine linker (1.2 equiv), 4,7-dichloroquinoline/4-chloroquinoline (1 equiv), and phenol (15 equiv) were heated at 120–130 °C, with stirring for 24 h or the reaction mixture was subjected to MW irradiation using a Biotage Initiator 2.5 apparatus. The reaction mixture was colled to rt and taken up in CH_2Cl_2. The organic layer was successively washed several times with NaOH and finally with brine. The organic layer was dried over anhydrous Na_2SO_4, and solvent was removed under reduced pressure to get a final product.

*General Procedure for Palladium Catalyzed Amination of Quinolines (**12, 24–26, 32, 37–39, 44–45, and 69–76**).*[62] Vial was charged with mixture of $Pd(OAc)_2$ (4 mol %) and DPEphos (8 mol %)/SPhos (8 mol %) in dioxane and stirred for a few minutes in Ar at rt. Subsequently, a haloquinoline (1.0 equiv), amine (1.2 equiv), and K_3PO_4 (2.5 equiv) were added to the reaction mixture. Resulting suspension was flushed with argon for several minutes. The vial was quickly capped, heated to 85 °C overnight, and then cooled down to rt. The mixture was adsorbed onto silica gel and purified.

*General Procedure for N-Methylation of Aminoquinolines (**47–48, 50–51**).*[63] To a stirred solution of aminoquinolines (1equiv) in MeOH containing 37% aqueous formaldehyde/acetone (2 equiv) was added mixture of $ZnCl_2$ (2 equiv) and $NaHB_3CN$ (4 equiv) in MeOH. After the reaction mixture was stirred at rt for 4 h, the solution was taken up in 0.1 M NaOH, and most of methanol was evaporated under reduced pressure. Aqueous solution was extracted with CH_2Cl_2, and the combined extracts were washed with water and brine, dried over anhyd Na_2SO_4, and the solvent was evaporated under reduced pressure.

*7-Chloro-3-fluoroquinoline (**15**).* The mixture of amine **13** (500 mg, 2.80 mmol) in 48% tetrafluoroboric acid (730 μL, 5.60 mmol) was stirred on ice bath. A solution of sodium nitrite (193 mg, 2.80 mmoL) in water (1 mL) was added dropwise after 15 min. The reaction mixture was stirred as the temperature rose to rt, then filtered under reduced pressure to separate the yellow precipitate, diazonium tetrafluoroborate, which was washed with cold EtOH and Et_2O and then dried under reducd pressure. Diazonium salt was suspended in 1,2-dichlorobenzene, and the reaction mixture was heated to 110 °C for 2 h. 7-Chloro-3-fluoroquinoline (**15**) was obtained after dry-flash chromatography (SiO_2, eluent Hex/EtOAc = 9/1). **15**: Light-yellow solid softens at 76–80 °C (Hex); yield 356 mg (70%). IR (ATR): 3076m, 3053m, 2957w, 2857w, 1615m, 1596s, 1565m, 1485m, 1443m,

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1414w, 1359w, 1333s, 1263m, 1184s, 1149w, 1129w, 1094w, 1071m, 988m, 961w, 935w, 913m, 891s, 812m, 776m, 645m, 537m, 512m, 477m, 461m cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.81 (d, J = 2.8, H-C(2)), 8.11 (d, J = 1.8, H-C(8)), 7.80−7.70 (m, 2H, H-C(4) and H−C(5)), 7.55−7.50 (m, H-C(6)). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 156.25 (d, J = 257.0, C(3)), 145.60 (C(8a)), 142.52 (d, J = 27.2, C(2)), 134.41 (C(7)), 128.73 (C(6)), 128.53 (C(5)), 129.20 (C(4a)), 128.35 (d, J = 5.1, C(8)), 118.31 (d, J = 17.3, C(4)). HRMS: m/z 182.01646 corresponds to molecular formula $C_9H_5ClFNH^+$ (error in ppm: −1.49).

*3-Fluoroquinoline (**16**).* Compound **16** was prepared according to the procedure described for compound **15**, starting from 3-aminoquinoline (**14**) (3 g, 20.96 mmol). 3-Fluoroquinoline (**16**) was obtained after chromatography (SiO_2, eluent Hex/EtOAc = 9/1) as light-yellow solid (4.90 g, 97%). IR (ATR): 3414w, 3064m, 2927w, 1652w, 1612s, 1560w, 1498s, 1463s, 1426m, 1371w, 1339s, 1213s, 1155s, 984m, 957w, 894m, 859w, 782m, 735m, 710w, 611w, 472w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.82 (d, J = 2.8, H-C(2)), 8.13 (d, J = 8.5, H-C(8)), 7.85−7.75 (m, 2H, H-C(4) and H-C(5)), 7.75−7.65 (m, H-C(7)), 7.65−7.55 (m, H-C(6)). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 156.25 (d, J = 255.0, C(3)), 145.40 (C(8a)), 141.54 (d, J = 27.6, C(2)), 129.49 (C(8)), 128.54 (C(7)), 128.42 (d, J = 5.7, C(4a)), 127.67 (C(6)), 127.27 (d, J = 4.6, C(5)), 118.32 (d, J = 17.2, C(4)). HRMS: m/z 148.05503 corresponds to molecular formula $C_9H_6FNH^+$ (error in ppm: −4.52).

*7-Chloro-3-fluoro-4-iodoquinoline (**17**).* Diisopropylamine (1.8 mL, 12.89 mmol) was added to *n*-butyllithium in hexane (4.9 mL, 12.28 mmol) in THF (6.5 mL) at −78 °C. The stirring continued for 30 min, and a solution of **15** (2.23 g, 12.28 mmol) in 5.5 mL of THF was slowly added to formed LDA solution at −85 °C. The resulting reaction mixture was stirred for 4 h at −78 °C, and iodine (3.40 g, 13.51 mmol) in 6 mL of THF was slowly added at −85 °C. Stirring was continued for 2 h at −78 °C before hydrolysis by 10 mL of THF/H_2O = 9/1. Extraction with EtOAc, drying over anhyd Na_2SO_4, filtration, and solvent removal afforded crude product which was purified by multiple chromatography: dry-flash (SiO_2, gradient Hex/EtOAc = 95/5→9/1) and flash chromatography (Biotage SP1 NH column, eluent: Hex/EtOAc = 9/1). **17**: Yield 1.53 g (41%). IR (ATR): 3080m, 3031w, 2959w, 2925w, 1837w, 1738w, 1602m, 1585s, 1551s, 1481s, 1441s, 1384w, 1357s, 1317s, 1307s, 1254m, 1210m, 1179m, 1139m, 1075m, 1018w, 949w, 925s, 895w, 870m, 851w, 814m, 755m, 642w, 576w, 536m, 509m, 429w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.60 (s, H-C(2)), 8.11 (d, J = 2.0, H-C(8)), 7.97 (d, J = 8.9, H-C(5)), 7.65−7.60 (m, H-C(6)). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 157.11 (d, J = 255.3, C(3)), 145.36 (C(8a)), 140.80 (d, J = 30.0, C(2)), 135.37 (C(7)), 132.16 (C(5)), 129.93 (C(6)), 129.20 (C(4a)), 128.63 (C(8)), 94.90 (d, J = 23.0, C(4)). HRMS: m/z 307.91326 corresponds to molecular formula $C_9H_4ClFINH^+$ (error in ppm: −0.36).

The compound **18** was also isolated as side product (359 mg, 9.5%).

*7-Chloro-3-fluoro-2-iodoquinoline (**18**).* IR (ATR): 3171w, 3071w, 3037s, 2923s, 2853m, 1729w, 1689w, 1603m, 1481m, 1404m, 1373w, 1330s, 1297w, 1255w, 1191s, 1135w, 1071w, 1016m, 918m, 866w, 815w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.08 (d, J = 1.8, H-C(8)), 7.72 (d, J = 8.7, H-C(5)), 7.64 (d, J = 7.1, H-C(4)), 7.55 (dd, J_1 = 2.0, J_2 = 8.8, H−C(6)). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 154.77 (d, J = 256.0, C(3)), 146.73 (C(8a)), 135.35 (C(7)), 129.27 (C(6)), 128.21 (d, J = 20.0, C(5)), 127.94 (C(8)), 126.36 (C(4a)), 117.50 (d, J = 85.2, C(4)), 111.95 (d, J = 120.1, C(2)). HRMS: m/z 307.91325 corresponds to molecular formula $C_9H_4ClFINH^+$ (error in ppm: −0.40).

*3-Fluoro-4-iodoquinoline (**19**).*[64] Compound **19** was prepared according to the procedure described for compound **17**, starting from 3-fluoroquinoline (1 g, 6.80 mmol). The crude product was purified by dry-flash chromatography (SiO_2, gradient Hex/EtOAc = 95/5 → 9/1). **19**: Yield 1.30 g (70%). IR (ATR): 3069m, 2924m, 2854w, 1586s, 1558s, 1491s, 1455s, 1414m, 1382w, 1339s, 1306s, 1262m, 1213s, 1141s, 1011w, 959w, 920m, 797w, 754s, 723m, 630w, 506w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.61 (s, H-C(2)), 8.09 (dd, J_1 = 0.6, J_2 = 8.1, H-C(8)), 8.02 (dd, J_1 = 1.5, J_2 = 8.4, H-C(5)), 7.75−7.70 (m, H-C(7)), 7.70−7.62 (m, H-C(6)). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 157.02 (d, J = 252.7, C(3)), 145.33 (d, J = 1.8, C(8a)), 139.85 (d, J = 30.7, C(2)), 130.87 (d, J = 5.4, C(5)), 130.62 (C(4a)), 129.86 (C(8)), 129.15 (d, J = 2.7, C(7)), 129.07 (C(6)), 95.19 (d, J = 21.7, C(4)). HRMS: m/z 273.95195 corresponds to molecular formula $C_9H_5FINH^+$ (error in ppm: −1.46).

*N^2-[2-(1-Adamantyl)ethyl]-N^1-(7-chloroquinolin-4-yl)propane-1,2-diamine (**34**).* Compound **34** was prepared from amine linker **S32** (140 mg, 0.59 mmol) and 4,7-dichloroquinoline (97 mg, 0.49 mmol) in phenol (692 mg, 7.35 mmol) and was isolated after multiple chromatography: dry-flash (SiO_2, eluent CH_2Cl_2/MeOH(NH_3 satd) = 95/5) and flash chromatography (Biotage SP1 NH column, eluent: Hex/EtOAc = 1/1) as colorless powder softening at 134−136 °C; yield 174 mg (74%). IR (ATR): 3245w, 2895s, 2842m, 1737m, 1670w, 1566s, 1489w, 1449m, 1427m, 1367m, 1331w, 1304w, 1284w, 1238m, 1202w, 1156w, 1136m, 1076m, 1046m, 989w, 966w, 897w, 869w, 843m, 804m, 769m, 704w, 646w, 622w, 594w, 570w, 544w, 524w, 517w, 509w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.52 (d, J = 5.3, H-C(2)), 7.95 (d, J = 2.1, H-C(8)), 7.70 (d, J = 8.9, H-C(5)), 7.36 (dd, J_1 = 2.2, J_2 = 8.9, H-C(6)), 6.36 (d, J = 5.4, H-C(3)), 6.01 (bs, H-NAr), 3.35−3.25 (m, 1H, ArNHCH_2CH(CH_3)−), 3.15−3.05 (m, 1H, ArNHCH_2CH(CH_3)−), 3.05−2.95 (m, 1H, ArNHCH_2CH(CH_3)−), 2.80−2.70 (m, 1H, −NHC$H_2$$CH_2$Ad), 2.65−2.55 (m, 1H, −NHC$H_2$$CH_2$Ad), 1.94 (bs, 3H, −Ad), 1.75−1.55 (m, 6H, −Ad), 1.55−1.45 (m, 6H, −Ad), 1.35−1.25 (m, 3H, −NHCH$_2$CH_2Ad, *H*-N−), 1.22 (d, 3H, J = 6.0, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 152.12, 150.01, 149.19, 134.75, 128.76, 125.14, 121.25, 117.47, 99.20, 51.74, 47.22, 45.24, 42.77, 41.27, 37.10, 32.00, 28.64, 19.30. HRMS: m/z 398.23395 corresponds to molecular formula $C_{24}H_{32}ClN_3H^+$ (error in ppm: −4.53). Anal. ($C_{24}H_{32}ClN_3$) Calcd: C, 72.43; H, 8.10; N, 10.56. Found: C, 71.98; H, 8.49; N, 10.59. HPLC purity (λ = 330 nm): method A, RT 8.017 min, area 99.00%; method B, RT 9.696 min, area 98.87%.

*N^1-[2-(1-Adamantyl)ethyl]-N^2-(7-chloroquinolin-4-yl)propane-1,2-diamine (**33**).* Compound **33** was prepared from amine **S13** (180 mg, 0.76 mmol) and 1-adamantylacetaldehyde (136 mg, 0.76 mmol) using $NaBH(OAc)_3$ (58 mg, 1.52 mmol) and was isolated after dry-flash chromatography: (SiO_2, eluent CH_2Cl_2/MeOH(NH_3 satd) = 95/5 and flash chromatography (Biotage SP1 RP column, gradient: MeOH/H_2O = 7/3→9/1) as colorless solid (227 mg; 75%) softening at 46−47 °C. IR (ATR): 3252m, 3063w, 2896s, 2843m, 1610w, 1573s, 1537m, 1447m, 1426w, 1376m, 1330m, 1279w, 1241w, 1205w, 1134m, 1078w, 966w, 901w, 870m, 843w, 805m, 763m, 645w, 623w, 600w, 540w, 513w, 490w, 421w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 327 (10189), 253 (16143) nm. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.48 (d, J = 5.5, H-C(2)), 7.92 (d, J = 1.0, H-C(8)), 7.74 (d, J = 9.0, H-C(5)), 7.32 (dd, J_1 = 1.8, J_2 = 8.8, H-C(6)), 6.40 (d, J = 5.5, H-C(3)), 5.90 (br s, H-N), 3.75−3.65 (m, 1H, ArNHCH(CH_3)−), 2.95−2.85 (m, 2H, ArNHCH-(CH_3)CH_2−), 2.70−2.60 (m, 2H, −C$H_2$$CH_2$Ad), 1.92 (br s, 3H, −Ad), 1.75−1.55 (m, 6H, −Ad), 1.48 (br s, 6H, −Ad), 1.35−1.20 (m, 6H, −CH_2Ad, CH_3, *H*-N−). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 151.82, 149.31, 149.13, 134.75, 128.46, 125.04, 121.44, 117.55, 99.31, 54.36, 47.41, 44.59, 44.44, 42.62, 37.03, 31.86, 28.58, 18.11. HRMS: m/z 398.23435 corresponds to molecular formula $C_{24}H_{32}ClN_3H^+$ (error in ppm: −3.51). Anal. ($C_{24}H_{32}ClN_3$·1/2H_2O) Calcd: C, 70.83; H, 8.17; N, 10.32. Found: C, 70.44; H, 7.91; N, 10.27. HPLC purity (λ = 330 nm): method A, RT 7.891 min, area 97.79%; method B, RT 9.904 min, area 95.06%.

*N^1-(1-Adamantylmethyl)-N^3-(7-chloroquinolin-4-yl)butane-1,3-diamine (**23**).* Compound **23** was prepared from amine **S14** (430 mg, 1.72 mmol) and adamantane-1-carboxaldehyde (283 mg, 1.72 mmol) using AcOH (141 μL, 2.15 mmol) and $NaBH_4$ (390 mg, 10.32 mmol) and was obtained after dry-flash chromatography: (SiO_2, eluent CH_2Cl_2/MeOH(NH_3 satd) = 95/5) as colorless foam (610 mg, 89%) softening at 135−138 °C. IR (ATR): 3250m, 3061w, 2962w, 2897s, 2842m, 1611w, 1570s, 1542m, 1490w, 1450m, 1430m, 1365m, 1345w, 1330m, 1283w, 1253w, 1206w, 1184w, 1136w, 1079w, 928w, 901w, 870m, 850m, 821w, 801m, 770w, 756m, 637w, 612w, 602w, 502w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 326 (10000), 254 (16260) nm. ^1H NMR (500 MHz,

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$CDCl_3$, δ): 8.49 (d, J = 5.4, H-C(2)), 7.92 (d, J = 2.0, H-C(8)), 7.80 (d, J = 8.9, H-C(5)), 7.35−7.20 (m, H-C(6)), 7.08 (d, J = 5.1, H-N), 6.40 (d, J = 5.4, H-C(3)), 3.90−3.80 (m, 1H, ArNHC*H*(CH_3)−), 2.95−2.85 (m, 1H, −CH_2NHCH_2Ad), 2.85−2.70 (m, 1H, −CH_2NHCH_2Ad), 2.30 (s, 2H, −CH_2Ad), 2.01 (s, 3H, −Ad), 1.95−1.85 (m, 1H, ArNHCH(CH_3)CH_2−), 1.85−1.65 (m, 7H, ArNHCH(CH_3)CH_2−, −Ad), 1.65−1.50 (m, 7H, −Ad, *H*-N−), 1.32 (d, 3H, J = 6.3, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 152.05, 149.61, 149.40, 134.59, 128.60, 124.54, 122.30, 117.67, 98.90, 63.68, 48.32, 47.81, 41.16, 37.17, 35.22, 33.36, 28.43, 19.36. HRMS: m/z 398.23673 corresponds to molecular formula $C_{24}H_{32}ClN_3H^+$ (error in ppm: + 2.45). Anal. ($C_{24}H_{32}ClN_3$·1/2H_2O) Calcd: C, 70.83; H, 8.17; N, 10.32. Found: C, 70.71; H, 7.91; N, 10.53. HPLC purity (λ = 330 nm): method A, RT 7.989 min, area 99.68%; method B, RT 9.636 min, area 99.31%.

*N^1-(1-Adamantylmethyl)-N^3-quinolin-4-ylbutane-1,3-diamine (**24**).* Compound **24** was prepared from amine linker **S52** (120 mg, 0.64 mmol) and 4-chloroquinoline (70 mg, 0.43 mmol) using $Pd(OAc)_2$ (3.79 mg 0.017 mmol), SPhos (13.88 mg, 0.036 mmol), and K_3PO_4 (224 mg, 1.08 mmol) and was obtained after dry-flash chromatography (SiO_2, gradient EtOAc/[MeOH/(NH_3 aq) = 9/1] = 9/1 → 7/3) as light-yellow foam softening at 160−161 °C; yield 114 mg (73%). IR (ATR): 3270m, 3070m, 2965m, 2902s, 2846s, 1618s, 1580w, 1539s, 1451s, 1396m, 1395m, 1373w, 1340m, 1264m, 1151m, 1126m, 1103w, 809m, 763 m, 738m, 703w cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.51 (d, J = 5.3, H-C(2)), 7.95 (dd, J_1 = 0.8, J_2 = 8.4, H-C(8)), 7.88 (dd, J_1 = 0.8, J_2 = 8.4, H-C(5)), 7.65−7.55 (m, H-C(7)), 7.40−7.30 (m, H-C(6)), 6.87 (br s, H-N), 6.42 (d, J = 5.6, H-C(3)), 3.95−3.80 (m, 1H, ArNHC*H*(CH_3)−), 2.95−2.85 (m, 1H, −CH_2NHCH_2Ad), 2.80−2.70 (m, 1H, −CH_2NHCH_2Ad), 2.29 (s, 2H, −NHCH_2Ad), 2.05−1.95 (m, 3H, −Ad), 1.95−1.85 (m, 1H, ArNHCH(CH_3)CH_2−), 1.84−1.60 (m, 8H, ArNHCH(CH_3)CH_2−, −Ad, *H*-N−), 1.58 (m, 6H, −Ad), 1.32 (d, 3H, J = 6.3, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 150.88, 149.60, 148.43, 129.56, 128.87, 124.00, 120.55, 119.14, 98.58, 63.65, 48.12, 47.83, 41.10, 37.17, 35.46, 33.44, 28.44, 19.15. HRMS: m/z 364.27556 corresponds to molecular formula $C_{24}H_{33}N_3H^+$ (error in ppm: + 2.30). Anal. ($C_{24}H_{33}N_3$·1/2H_2O) Calcd: C, 77.37; H, 9.20; N, 11.28. Found: C, 77.37; H, 9.03; N, 11.18. HPLC purity (λ = 330 nm) method A, RT 0.953 min, area 99.05%; method B, RT 9.386 min, area 97.27%.

*N^1-(1-Adamantylmethyl)-N^3-(7-chloro-3-fluoroquinolin-4-yl)-butane-1,3-diamine (**25**).* Compound **25** was prepared from amine linker **S52** (135 mg, 0.57 mmol) and **17** (159.64 mg, 0.52 mmol) using $Pd(OAc)_2$ (5.2 mg, 0.023 mmol), SPhos (18.88 mg, 0.046 mmol), and K_3PO_4 (303 mg, 1.43 mmol) and was obtained after multiple chromatography: dry-flash (SiO_2, gradient CH_2Cl_2/MeOH-(NH_3 satd) = 95/5 → 8/2) and flash chromatography (Biotage SP1 NH column, eluent Hex/EtOAc = 7/3; RP column, eluent MeOH/H_2O = 8/2) as colorless viscous oil (101.7 mg, 47%). IR (ATR): 3280m, 3070w, 2902s, 2846s, 2674w, 1596s, 1572s, 1540m, 1486w, 1450m, 1422m, 1380m, 1346m, 1295w, 1262m, 1191m, 1148m, 1115m, 1078m, 983w, 927m, 902w, 879m, 813m, 761w, 737m, 656w, 539w, 426w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 250 (15462), 337 (9685) nm. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.48 (d, J = 5.8, H-C(2)), 7.92 (d, J = 1.6, H-C(8)), 7.86 (d, J = 9.0, H-C(5)), 7.35−7.20 (m, H-C(6)), 6.63 (d, J = 4.6, H-N), 4.40−4.25 (m, 1H, ArNHC*H*(CH_3)−), 3.00−2.85 (m, 1H, −CH_2NHCH_2Ad), 2.85−2.70 (m, 1H, −CH_2NHCH_2Ad), 2.30 (s, 2H, −CH_2Ad), 2.05−1.90 (m, 4H, −Ad, ArNHCH(CH_3)CH_2−), 1.90−1.60 (m, 8H, ArNHCH(CH_3)CH_2−, −Ad, *H*-N), 1.60−1.50 (m, 6H, −Ad), 1.30 (d, 3H, J = 6.2, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 146.60 (C(8a)), 143.19 (d, J = 239.1, C(3)), 142.46 (d, J = 27.5, C(2)), 136.12 (d, J = 5.4, C(4)), 133.76 (d, J = 7.2, C(7)), 128.63 (C(8)), 125.37 (C(6)), 122.94 (d, J = 5.0, C(5)), 119.64 (d, J = 5.4, C(4a)), 50.58 (d, J = 8.6, ArNHCH(CH_3)−), 47.52, 41.05, 37.08, 35.75, 33.30, 28.36, 21.60. HRMS: m/z 416.22602 corresponds to molecular formula $C_{24}H_{31}ClFN_3H^+$ (error in ppm: −0.75). Anal. ($C_{24}H_{31}ClFN_3$·2/3H_2O) Calcd: C, 67.35; H, 7.61; N, 9.82. Found: C, 67.17; H, 7.47; N, 9.74. HPLC purity (λ = 330 nm) method A, RT 8.589 min, area 97.89%; method B, RT 11.009 min, area 97.73%.

*N^1-(1-Adamantylmethyl)-N^3-(3-fluoroquinolin-4-yl)butane-1,3-diamine (**26**).* Compound **26** was prepared from amine linker **S52** (880 mg, 3.72 mmol) and **19** (1 g, 3.72 mmol) using $Pd(OAc)_2$ (33.4 mg, 0.15 mmol), DPEphos (160 mg, 0.30 mmol), and K_3PO_4 (1.97 g, 9.14 mmol) and was obtained after multiple chromatography: dry-flash (SiO_2, gradient CH_2Cl_2/(NH_3 satd) 95/5 → 7/3) and flash chromatography (Biotage SP1 NH column, eluent Hex/EtOAc = 7/3. and RP column, gradient MeOH/H_2O = 8/2 → 9/1) as colorless oil (572 mg, 49%). IR (ATR): 3265m, 2902s, 2845m, 1601s, 1574s, 1540s, 1496w, 1451m, 1398m, 1366w, 1345w, 1270w, 1202w, 1148w, 759m cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.51 (d, J = 5.7, H-C(2)), 8.00−7.88 (m, H-C(8) and H-C(5)), 7.60−7.54 (m, H-C(7)), 7.41−7.35 (m, H-C(6)), 6.31 (s, H-N), 4.37−4.27 (m, 1H, ArNHC*H*(CH_3)−), 2.95−2.85 (m, 1H, −CH_2NHCH_2Ad), 2.85−2.75 (m, 1H, −CH_2NHCH_2Ad), 2.30 (s, 2H, −CH_2Ad), 2.01−1.96 (m, 3H, −Ad), 1.80−1.60 (m, 9H, ArNHCH(CH_3)CH_2−, −Ad, *H*-N−), 1.60−1.50 (m, 6H, −Ad), 1.30 (dd, J_1 = 0.9, J_2 = 6.42, −CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 146.01 (C(8a)), 143.22 (d, J = 238.3, C(3)), 141.60 (d, J = 27.1, C(2)), 135.80 (d, J = 5.4, C(4)), 129.85 (C(8)), 127.90 (C(7)), 124.83 (C(6)), 124.68 (C(4a)), 121.27 (d, J = 5.4, C(5)), 63.70, 50.44 (d, J = 8.1, CH), 41.06, 37.15, 36.21, 33.33, 28.42, 21.71 (d, J = 2.7, CH_3). HRMS: m/z 382.26498 corresponds to molecular formula $C_{24}H_{32}FN_3H^+$ (error in ppm: −0.83). HPLC purity (λ = 330 nm) method A, RT 7.545 min, area 99.28%; method B, RT 8.251 min, area 99.45%.

*N^1-[2-(1-Adamantyl)ethyl]-N^4-(7-chloroquinolin-4-yl)pentane-1,4-diamine (**42**).* Compound **42** was prepared from amine **S15** (320 mg, 1.21 mmol) and adamantane-1-carboxaldehyde (216 mg, 1.21 mmol) using AcOH (86 μL, 1.51 mmol) and $NaBH_4$ (92 mg, 2.42 mmol) and was obtained after dry-flash chromatography: (SiO_2, eluent CH_2Cl_2/MeOH(NH_3 satd) = 9/1) as colorless foam (314 mg, 61%) softening at 49−51 °C. IR (ATR): 3422m, 2901s, 2845m, 1611w, 1578s, 1540w, 1450m, 1426w, 1379w, 1332w, 1280w, 1253w, 1201w, 1150w, 1081w, 905w, 877w, 854w, 806w, 768w, 646w, 601w, 401w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 328 (11161), 255 (17040) nm. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.50 (d, J = 5.4, H-C(2)), 7.93 (d, J = 2.1, H-C(8)), 7.73 (d, J = 9.0, H-C(5)), 7.33 (dd, J_1 = 2.2, J_2 = 8.9, H-C(6)), 6.39 (d, J = 5.5, H-C(3)), 5.52 (br s, H-N), 3.75−3.60 (m, 1H, ArNHC*H*(CH_3)−), 2.75−2.65 (m, 2H, −CH_2NHCH_2CH$_2$Ad), 2.65−2.55 (m, 2H, −CH_2CH$_2$Ad), 1.93 (br s, 3H, −Ad), 1.85−1.75 (m, 1H, ArNHCH(CH_3)CH_2−), 1.75−1.50 (m, 10H, ArNHCH-(CH_3)CH_2−, −CH_2CH$_2$NHCH_2CH$_2$Ad, −Ad, *H*-N−), 1.45 (m, 6H, −Ad), 1.35−1.25 (m, 5H, CH_3, −CH_2Ad). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 151.95, 149.32, 149.11, 134.72, 128.68, 124.96, 121.35, 117.35, 99.12, 49.57, 48.30, 44.48, 44.33, 42.60, 37.08, 34.05, 31.82, 28.61, 26.33, 20.16. HRMS: m/z 426.26752 corresponds to molecular formula $C_{26}H_{36}ClN_3H^+$ (error in ppm: + 1.10). Anal. ($C_{26}H_{36}ClN_3$·3/2H_2O) Calcd: C, 68.93; H, 8.68; N, 9.27. Found: C, 69.08; H, 9.04; N, 9.48. HPLC purity (λ = 330 nm): method A, RT 8.000 min, area 95.37%; method B, RT 9.821 min, area 95.54%.

*N^4-(7-Chloro-3-fluoroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine (**74**).* Compound **74** was prepared from **17** (300 mg, 0.98 mmol) and 2-amino-5-diethylaminopentane (378 μL, 1.95 mmol) using $Pd(OAc)_2$ (8.8 mg, 0.039 mmol), DPEphos (42 mg, 0.078 mmol), and K_3PO_4 (518 mg, 2.44 mmol) and was obtained after multiple chromatography: dry-flash (SiO_2, gradient CH_2Cl_2/MeOH-(NH_3 satd) = 95/5 → 8/2) and flash chromatography (Biotage SP1 NH column, eluent Hex/EtOAc = 8/2) as colorless foam (329 mg, 54%) softening at 48−50 °C. IR (ATR): 3304m, 3069w, 2970s, 2936m, 2871w, 2810w, 1595s, 1574s, 1536m, 1488w, 1452m, 1418m, 1380m, 1351m, 1294w, 1265m, 1221w, 1197w, 1140m, 1078m, 929w, 906w, 877w, 815m, 764w, 735w, 656w, 540w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 336 (11554), 252 (16869) nm. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.52 (d, J = 5.5, H-C(2)), 7.96 (d, J = 2.2, H-C(8)), 7.75 (d, J = 9.1, H-C(5)), 7.42−7.36 (m, H-C(6)), 4.73 (d, J = 7.7, −N*H*), 4.20−4.05 (m, 1H, ArNHC*H*(CH_3)−), 2.54 (q, 4H, J = 7.2, −NH(CH_2CH$_3$)$_2$), 2.50−2.43 (m, 2H, −CH_2NH(CH$_2$CH$_3$)$_2$), 1.75−1.55 (m, 4H, ArNHCH-(CH_3)CH_2−, ArNHCH(CH_3)CH$_2$CH_2−), 1.29 (dd, 3H, J_1 = 0.9, J_2 = 6.3, ArNHCH(CH_3)−), 1.01 (t, 6H, J = 7.0, −NH(CH$_2$CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 146.62 (C(8a)), 143.42 (d, J = 240.1,

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C(3)), 142.45 (d, J = 27.3, C(2)), 135.77 (d, J = 6.3, C(4)), 133.97 (C(7)), 128.96 (C(8)), 125.95 (C(6)), 121.89 (d, J = 5.4, C(5)), 119.45 (d, J = 4.5, C(4a)), 52.54, 51.34 (d, J = 8.1, ArNHCH(CH_3)−), 46.78, 36.12, 23.70, 22.30, 11.26. HRMS: m/z 338.18020 corresponds to molecular formula $C_{18}H_{25}ClFN_3H^+$ (error in ppm: + 2.43. Anal. ($C_{18}H_{25}ClFN_3 \cdot 1/2H_2O$) Calcd: C, 62.33; H, 7.56; N, 12.11. Found: C, 62.00; H, 7.32; N, 11.85. HPLC purity (λ = 330 nm): method A, rt 7.677 min, area 98.24%; method B, RT 9.229 min, area 98.45%.

*N′-(7-Chloro-3-fluoroquinolin-4-yl)-N,N-diethylpropane-1,3-diamine (**73**).* Compound **73** was prepared from **17** (100 mg, 0.32 mmol) and 3-diethylamino-1-propilamine (105 μL, 0.65 mmol) using $Pd(OAc)_2$ (2.9 mg, 0.013 mmol), SPhos (10.7 mg, 0.026 mmol), and K_3PO_4 (178 mg, 0.81 mmol) and was obtained after multiple chromatography: dry-flash (SiO_2, gradient CH_2Cl_2/MeOH(NH_3 satd) = 95/5 → 8/2) and flash chromatography (Biotage SP1 NH column, eluent Hex/EtOAc = 85/15) as colorless foam (64 mg, 63%) softening at 47−46 °C. IR (ATR): 3234m, 3065w, 2970s, 2934m, 2872w, 2822m, 1640w, 1597s, 1574s, 1540m, 1489w, 1470w, 1454w, 1429m, 1380m, 1360m, 1294w, 1268w, 1246w, 1195m, 1166w, 1138m, 1077m, 934m, 892m, 812m, 762m, 737w, 654w, 622w, 540w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 337 (9690), 252 (15073) nm. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.45 (d, J = 6.0, H-C(2)), 7.91 (d, J = 2.2, H-C(8)), 7.75 (d, J = 9.0, H-C(5)), 7.32 (dd, J_1 = 1.6, J_2 = 9.0, H-C(6)), 3.90−3.80 (m, 2H, ArNHCH_2−), 2.72−2.68 (m, 2H, −CH_2NH(CH_2CH_3)$_2$), 2.65 (q, 4H, J = 7.1, −NH(C$H_2$$CH_3$)$_2$), 2.04 (br s, H-N), 1.90−1.80 (m, 2H, ArNHCH$_2$CH_2−), 1.10 (t, 6H, J = 7.1, −NH(CH_2CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$): 146.60 (C(8a)), 143.50 (d, J = 226.3, C(3)), 142.45 (d, J = 27.2, C(2)), 137.28 (d, J = 5.9, C(4)), 133.72 (d, J = 7.2, C(7)), 128.65 (C(8)), 125.23 (C(6)), 122.74 (d, J = 5.0, C(5)), 119.28 (d, J = 5.9, C(4a)), 53.50, 47.44 (d, J = 9.9, ArNHCH$_2$−), 47.03, 25.31, 11.28. HRMS: m/z 310.14830 corresponds to molecular formula $C_{16}H_{21}ClFN_3H^+$ (error in ppm: + 0.71). (+)ESI-HRMS (m/z (%)): 310.14830 ([M + H]$^+$, 100); calculated 310.14808 (error in ppm: 0.71). Anal. ($C_{16}H_{21}ClFN_3 \cdot 1/2H_2O$) Calcd: C, 60.28; H, 6.96; N, 13.18. Found: C, 60.65; H, 7.07; N, 12.79. HPLC purity (λ = 330 nm): method A, RT 7.585 min, area 99.71%; method B, RT 8.693 min, area 95.30%.

*N′-(7-Chloro-3-fluoroquinolin-2-yl)-N,N-diethylpropane-1,3-diamine (**75**).* Compound **75** was prepared from 7-chloro-3-fluoro-2-iodoquinoline **18** (100 mg, 0.32 mmol) and 3-diethylamino-1-propilamine (74 μL, 0.46 mmol) using $Pd(OAc)_2$ (2.0 mg, 0.009 mmol), SPhos (7.5 mg, 0.018 mmol), and K_3PO_4(124 mg, 0.57 mmol) and was obtained after multiple chromatography: dry-flash (SiO_2, gradient CH_2Cl_2/MeOH(NH_3 satd) = 95/5 → 8/2) and flash chromatography (Biotage SP1 RP column, eluent MeOH/H_2O = 8/2) as a yellow viscous oil (51 mg, 72%). IR (ATR): 3450w, 3241m, 2970m, 2931m, 2873w, 2815m, 1639m, 1610w, 1563w, 1537s, 1497w, 1459m, 1416m, 1378w, 1338m, 1292m, 1256m, 1193m, 1146m, 1124m, 1098w, 1070m, 1032w, 979w, 921w, 885m, 800w, 761w, 739w, 604w, 514w, 476w cm^{-1}. $\lambda_{max}(\varepsilon)$ = 343 (8682), 332 (8760), 242 (35685) nm. ^1H NMR (500 MHz, $CDCl_3$, δ): 7.69 (d, J = 2.0, H-C(8)), 7.50−7.40 (bs, 1H, H-N−), 7.41 (d, J = 8.5, H-C(5)), 7.33 (d, J = 11.2, H-C(4)), 7.14 (dd, J_1 = 2.0, J_2 = 8.4, H-C(6)), 3.75−3.60 (m, 2H, ArNHCH_2−), 2.64 (t, 2H, J = 6.0, −CH_2NH(CH_2CH_3)$_2$), 2.57 (q, 4H, J = 7.1, −NH(C$H_2$$CH_3$)$_2$), 1.90−1.75 (m, 2H, ArNHCH$_2$CH_2−), 1.07 (t, 6H, J = 7.2, −NH(CH_2CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 149.16 (d, J = 14.0, C(8a)), 147.58 (d, J = 259.0, C(3)), 145.54 (d, J = 2.7, C(2)), 133.77 (d, J = 7.2, C(7)), 127.77 (d, J = 5.0, C(5)), 125.03 (C(8)), 122.80 (C(6)), 121.26 (d, J = 4.5, C(4a)), 115.56 (d, J = 15.4, C(4)), 53.00, 46.83, 41.57, 25.11, 11.53. HRMS: m/z 310.14742 corresponds to molecular formula $C_{16}H_{21}ClFN_3H^+$ (error in ppm: −2.14). Anal. ($C_{16}H_{21}ClFN_3 \cdot 1/4H_2O$) Calcd: C, 61.14; H, 6.89; N, 13.37. Found: C, 61.33; H, 6.96; N, 13.40. HPLC purity (λ = 330 nm): method A, RT 7.559 min, area 99.45%; method B, rt 8.647 min, area 99.28%.

*N-(7-Chloroquinolin-4-yl)-N′-[(5-fluoro-1-benzothiophen-3-yl)methyl]propane-1,3-diamine (**58**).* Compound **58** was prepared from aldehyde **S75** (181 mg, 1.00 mmol) using amine **AQ3** (353 mg, 1.50 mmol). The product was purified using column chromatography (dry-flash, SiO_2, eluent EtOAc/Hex gradient 1/9 → EtOAc, EtOAc/MeOH gradient 95/5 → 4/6, flash, Biotage SP1, NH column, eluent EtOAc/Hex gradient 8/2 → EtOAc, EtOAc/MeOH gradient 95/5 → MeOH, and flash, Biotage SP1, SiO_2 column, eluent EtOAc/MeOH + NH_3(9/1) gradient 95/5 → 7/3). Final product **58** was obtained as colorless powder (269 mg, 67%); mp = 133 − 134 °C. IR (ATR): 3240m, 3060w, 2953w, 2852w, 1607m, 1579s, 1535m, 1435m, 1360w, 1329m, 1279w, 1251w, 1230w, 1205w, 1137w, 1104w, 1082w, 913w, 850w, 800w, 761w, 718w, 665w, 642w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.50−8.47 (m, H-C(2′)), 7.90−7.88 (m, H-C(8′)), 7.83 (dd, J_1 = 4.8, J_2 = 8.9, H-C(7)), 7.46 (dd, J_1 = 2.4, J_2 = 9.5, H-C(4)), 7.43 (s, H-C(2)), 7.35 (d, J = 9.0, H-C(5′)), 7.17−7.09 (m, H-C(6) and H-N exchangeable with D_2O), 7.01−6.97 (m, H-C(6′)), 6.32 (d, J = 5.5, H-C(3′)), 4.06 (s, 2H, −CH_2NHCH$_2$CH$_2$CH$_2$NH−), 3.45−3.40 (m, 2H, −CH$_2$NHCH$_2$CH$_2$CH_2NH−), 3.06−3.02 (m, 2H, −CH$_2$NHCH_2CH$_2$CH$_2$NH−), 2.01−1.94 (m, 2H, −CH$_2$NHCH$_2$CH_2CH$_2$NH−), 1.69 (bs, H-N exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 160.88 (d, J = 240.1), 152.04, 150.24, 149.05, 139.37 (d, J = 9.0), 135.98, 134.48 (d, J = 22.5), 128.48, 125.89, 124.81, 124.17 (d, J = 9.9), 121.61, 117.36, 113.51 (d, J = 25.3), 107.28 (d, J = 22.5), 98.39, 49.36, 47.64, 43.68, 27.60. HRMS: m/z 400.10388 corresponds to molecular formula $C_{21}H_{19}ClFN_3SH^+$ (error in ppm −1.56). Anal. ($C_{21}H_{19}ClFN_3S$) Calcd: C, 63.07; H, 4.79; N, 10.51; S, 8.02. Found: C, 63.09; H, 4.77; N, 10.30; S, 7.72. HPLC purity (λ = 330 nm): method A, RT 8.998 min, area 98.73%; method B, RT 8.544 min, area 99.17%.

*N-(7-Chloroquinolin-4-yl)-N′-[(6-fluoro-1-benzothiophen-3-yl)methyl]propane-1,3-diamine (**59**).* Compound **59** was prepared from aldehyde **S76** (161 mg, 0.888 mmol) using amine **AQ3** (314 mg, 1.33 mmol). The product was purified using column chromatography (dry-flash, SiO_2, eluent EtOAc/Hex gradient 1/9 → EtOAc, EtOAc/MeOH gradient 95/5 → 4/6, flash, Biotage SP1, NH column, eluent EtOAc/Hex gradient 8/2 → EtOAc, EtOAc/MeOH gradient 95/5 → MeOH, and flash, Biotage SP1, SiO_2 column, eluent EtOAc/MeOH + NH_3(9/1) gradient 95/5 → 3/7). Final product **59** was obtained as colorless powder (194 mg, 54%); mp = 149−150 °C. IR (ATR): 3433w, 3294m, 3210m, 3067m, 3015m, 2927m, 2853m, 1606m, 1581s, 1539m, 1466m, 1430w, 1369w, 1330w, 1282w, 1251w, 1208w, 1168w, 1139w, 1109w, 1080w, 896w, 854w, 807w, 761w, 737w, 683w, 646w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.50−8.47 (m, H-C(2′)), 7.90 (bs, H-C(8′)), 7.74−7.70 (m, H-C(4)), 7.60−7.56 (m, H-C(7)), 7.37−7.33 (m, H-C(5′)), 7.30 (s, H-C(2)), 7.22 (bs, H-N exchangeable with D_2O), 7.07−7.01 (m, H-C(5)), 7.00−6.96 (m, H-C(6′)), 6.31 (d, J = 5.2, H-C(3′)), 4.08 (s, 2H, −CH_2NHCH$_2$CH$_2$CH$_2$NH−), 3.45−3.40 (m, 2H, −CH$_2$NHCH$_2$CH$_2$CH_2NH−), 3.06−3.03 (m, 2H, −CH$_2$NHCH_2CH$_2$CH$_2$NH−), 2.01−1.95 (m, 2H, −CH$_2$NHCH$_2$CH_2CH$_2$NH−), 1.76 (bs, H-N exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 160.70 (d, J = 243.7), 151.97, 150.31, 141.61 (d, J = 9.9), 134.71, 134.12, 128.39, 124.83, 122.98, 122.46 (d, J = 10.0), 121.70, 117.33, 113.28 (d, J = 23.5), 109.07 (d, J = 25.3), 98.36, 49.37, 47.69, 43.75, 27.52. HRMS: m/z 400.10427 corresponds to molecular formula $C_{21}H_{19}ClFN_3SH^+$ (error in ppm −0.56). HPLC purity (λ = 330 nm): method A, RT 9.049 min, area 95.99%; (λ = 254 nm) method B, RT 7.455 min, area 97.07%.

*N-(7-Chloroquinolin-4-yl)-N′-[(5-fluoro-1-benzothiophen-3-yl)methyl]butane-1,4-diamine (**63**).* Compound **63** was prepared from aldehyde **S75** (181 mg, 1.00 mmol) using amine **AQ4** (376 mg, 1.51 mmol). The product was purified using column chromatography (dry-flash, SiO_2, eluent EtOAc/Hex gradient 1/9 → EtOAc, EtOAc/MeOH gradient 95/5 → 1/1, flash, Biotage SP1, NH column, eluent EtOAc/Hex gradient 8/2 → EtOAc, EtOAc/MeOH gradient 95/5 → MeOH, and flash, Biotage SP1, SiO_2 column, eluent EtOAc/MeOH + NH_3(9/1) gradient 95/5 → 1/1). Final product **63** was obtained as a white powder (251 mg, 60%); mp = 110−113 °C. IR (ATR): 3228m, 3063w, 2945w, 2855w, 2810w, 1579s, 1543m, 1490w, 1433m, 1366w, 1330w, 1279w, 1245w, 1225w, 1196w, 1161w, 1134w, 1078w, 954w, 898w, 854w, 806w, 782w, 640w, 619w, 543w, 483w, 452w, 423w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.50 (d, J = 5.2, H-C(2′)), 7.94−7.91 (m, H-C(8′)), 7.77 (dd, J_1 = 4.8, J_2 = 8.7, H-C(7)), 7.59 (d, J = 8.9, H-C(5′)), 7.51−7.46 (m, H-C(4)), 7.38 (s, H-C(2)), 7.18−7.14 (m, H-

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C(6′)), 7.10 (td, J_1 = 2.4, J_2 = 8.8, H-C(6)), 6.36 (d, J = 5.3, H-C(3′)), 5.65 (bs, H-N exchangeable with D_2O), 4.00 (s, 2H, $-CH_2NHCH_2CH_2CH_2CH_2NH-$), 3.32−3.27 (m, 2H, $-CH_2NHCH_2CH_2CH_2CH_2NH-$), 2.80 (t, J = 6.6, 2H, $-CH_2NHCH_2CH_2CH_2CH_2NH-$), 1.88 (quin, 2H, J = 6.8, $-CH_2NHCH_2CH_2CH_2CH_2NH-$), 1.75−1.55 (m, 2H, $-CH_2NHCH_2CH_2CH_2CH_2NH-$ and H-N exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 160.74 (d, J = 240.1), 152.01, 149.82, 149.08, 139.46 (d, J = 9.0), 135.97, 134.81, 134.74 (d, J = 9.0), 128.68, 125.43, 125.02, 123.98 (d, J = 9.0), 121.05, 117.18, 113.29 (d, J = 24.4), 107.47 (d, J = 22.6), 98.91, 48.99, 47.67, 43.14, 27.69, 26.40. HRMS: m/z 414.11939 corresponds corresponds to molecular formula $C_{22}H_{21}ClFN_3SH^+$ (error in ppm −1.85). HPLC purity (λ = 330 nm): method A, RT 9.136 min, area 99.72%; (λ = 254 nm) method B, RT 7.522 min, area 95.22%.

Plasmodium Asexual Blood Stages. *In Vitro Antiplasmodial Activity.* The in vitro antimalarial drug susceptibility screen is a modification of the procedures previously published by Desjardins et al.[65] with modifications developed by Milhous et al.,[41] and full details are given in ref 42. All synthesized aminoquinolines were screened in vitro against the following *P. falciparum* strains: CQ and MFQ susceptible strain D6 (clone of SierraI/UNC isolate), CQ resistant but MFQ susceptible strain W2 (clone of Indochina I isolate), and CQ and MFQ resistant strain TM91C235 (clone of South-East Asian isolate).

Assessment of compound toxicity in a HepG2 (hepatocellular carcinoma) cells exactly followed the protocol described in detail.[66]

In Vivo Efficacy Studies. The *P. berghei* mouse efficacy tests were conducted using a modified version of the Thompson test. Briefly, groups of five mice were inoculated intraperitoneally with erythrocytes infected with *P. berghei* on day 0. Drugs were suspended in 0.5% hydroxyethylcellulose−0.1% Tween 80 and administered orally once a day beginning on day 3 post infection. Dosings are shown in Table 2. All untreated infected (control) mice showed parasitemia on day 3 and high parasite counts on day 6, with levels of parasitemia between 37.8% and 57.8% (mean 45.96%), succumbed to infection on day 6−8. Accordingly, the test was considered valid. Cure was defined as survival (with no parasitemia) until day 31 post-treatment. Parasitemia was determined by thin-blood Giemsa-stained smears prepared from mice tail blood of each animal on study days 0, 3, 6, 10, 13, 17, 20, 24, 27, and 31 (postinfection). The slides were examined under a light microscope.

In a separate host toxicity study, groups of five healthy mice were administered 160 mg/kg/day × 3 days of a compound under investigation. Monitoring individual mouse behavior and appearance two times a day for 31 days revealed no overt clinical manifestations of toxicity.

The study followed the International Guiding Principles for biomedical research involving animals, and was reviewed by a local Ethics Committee and approved by the Veterinary Directorate at the Ministry of Agriculture and Environmental Protection of Serbia (decision no. 323-07-02444/2014-05/1).

Antimalarial Activity against Stage IV−V *P. falciparum* Gametocytes. Gametocytes cultures were conducted as described recently.[50] The 3D7elo1-pfs16-CBG99 transgenic strain[67] expressing the CBG99 luciferase under the pfs16 gametocyte specific promoter was used in all the experiments, and the luciferase activity was taken as measure of gametocytes viability. Epoxomicin and dihydroartemisinin have been used as positive controls.

The IC_{50}, the dose which induces 50% inhibition of gametocytes viability, was extrapolated from the nonlinear regression analysis of the dose−response curve (software Gen5 1.10 provided with the Synergy4 plate reader, Biotek). The percentage of gametocytes viability was calculated as 100 × [(OD treated sample − OD blank)/(OD untreated sample − OD-blank)] where "blank" is the sample treated with 100 nM epoxomicin which completely kills gametocytes.

Liver Stage of *P. berghei* Infection. *In Vitro LS Activity.* Inhibition of liver-stage infection by test compounds was determined by measuring the luminescence intensity in Huh7 cells infected with a firefly luciferase-expressing *P. berghei* line, as previously described.[43] Briefly, Huh7 cells, a human hepatoma cell line, were cultured in 1640 rpmI medium supplemented with 10% v/v fetal bovine 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_2. For infection assays, Huh7 cells (1.0×10^4 per well) were seeded in 96-well plates the day before drug treatment and infection. The medium 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 1700*g* for 5 min. Parasite infection load was measured 48 h after infection by a bioluminescence assay (Biotium). The effect of the compounds on the viability of Huh7 cells was assessed by the AlamarBlue assay (Invitrogen, U.K.) using the manufacturer's protocol.

In Vivo Liver Stage Assay. C57Bl/6J mice were infected by intravenous inoculation of 5×10^3 luciferase-expressing *P. berghei* ANKA sporozoites freshly dissected from the salivary glands of infected *Anopheles stephensi* mosquitoes. The compound was administered by intraperitoneal injection 24 h prior to infection, at the time of infection, and 24 h after infection. An equivalent amount of drug vehicle was injected in control mice.

Determination of liver parasite loads in vivo was carried out as previously described, either by quantitative real-time PCR (qRT-PCR)[68] or by luminescence measurement.[43] For qRT-PCR analyses, livers were collected and homogenized in a denaturing solution (4 M guanidine thiocyanate; 25 mM sodium citrate [pH 7], 0.5% sarcosyl, and 0.7% β-mercaptoethanol in diethyl pyrocarbonate-treated water) 48 h after sporozoite injection. Total RNA was extracted using the Qiagen RNeasy mini kit according to the manufacturer's instructions. RNA for infection measurements was converted into cDNA by using the Nzytech kit according to the manufacturer's protocol. The quantitative real-time PCRs (qRT-PCRs) used the Applied Biosystems Power SYBR green PCR master mix and were performed according to the manufacturer's instructions on an ABI Prism 7500 Fast system (Applied Biosystems). Amplification reactions were carried out in a total reaction volume of 20 μL, using 1 μg cDNA and employing *P. berghei* ANKA 18S rRNA gene- or housekeeping gene-specific primers. Relative amounts of *P. berghei* ANKA mRNA were calculated against the amount of the hypoxanthine guanine phosphoribosyltransferase (HPRT) housekeeping gene. Primer sequences specific to each gene were as follows: for the *P. berghei* ANKA 18S rRNA gene, 5′-AAG CAT TAA ATA AAG CGA ATA CAT CCT TAC-3′ and 5′-GGA GAT TGG TTT TGA CGT TTA TGT G-3′; for the mouse HPRT gene, 5′-TGC TCG AGA TGT GAT GAA GG-3′ and 5′-TCC CCT GTT GAC TGG TCA TT-3′; and for the human HPRT gene, 5′-TGC TCG AGA TGT GAT GAA GG-3′ and 5′-TCC CCT GTT GAC TGG TCA TT-3′. For bioluminescence measurements, mice were infected with firefly luciferase-expressing *P. berghei* sporozoites and liver parasite loads were measured 46 h later on an IVIS Lumina system, following subcutaneous injection of the luciferin substrate.

Metabolic Stability Studies. *Liver Microsomal Preparations.* Selected samples were tested in human and mouse liver microsomal preparations exactly following the protocol described in detail.[38]

Hepatocyte Metabolic Stability (Performed at Cyprotex US, LLC, Watertown, MA). Compound **25** was incubated in duplicate with cryopreserved hepatocytes at 37 °C. The cells were thawed, viable cells counted, and equilibrated. After 30 min equilibration at 37 °C with gentle agitation, the test compounds were added into the cells to give the desired final concentration of 1 μM. The cell suspension was incubated at 37 °C as described above. At the indicated times, samples were removed and mixed with an equal volume of ice-cold stop solution (methanol containing internal analytical standards). Stopped reactions were incubated at least 10 min on ice, and an additional volume of water was added. The samples are centrifuged to remove precipitated protein, and the supernatants were analyzed by LC-MS/MS to monitor the parent depletion using the MRM method and known phase I and II metabolites (oxidation, demethylation, dehydrogenation, glucuronidation, and oxidation + glucuronidation)

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using SRM method. The amount of monitored **25** is converted to % remaining by dividing by the time zero concentration value. Data are fit to a first-order decay model to determine half-life. From a plot of log (ln) peak area against time, the slope of the line is determined. Half-life and intrinsic clearance are calculated using the equations below:

$$\text{elimination rate constant}(k) = (-\text{slope});\ \text{half-life}(T_{1/2})\ \text{min}$$

$$= 0.693/\text{k};\ \text{intrinsic clearance}(\text{CLint})(\text{mL/min /million cells})$$

$$= (V \times 0.693)/T_{1/2}$$

where V = incubation volume mL/number of cell. Controls: Verapamil, intrinsic clearance 11.9 and 29.5 μL/min/10^6 cells (human (h), mouse (m), respectively). Warfarin, intrinsic clearance <1.4 and <1.4 μL/min/10^6 cells (human (h), mouse (m), respectively).

β-Hematin Inhibitory Activity Assay. The inhibition of β-hematin formation is expressed as the molar equivalent of compound, relative to hemin, that inhibits β-hematin formation by 50% and determined by slightly modified BHIA assay introduced by Parapini et al.[57] Briefly, 50.0 μL of 16 mM solution of hemin in DMSO was distributed in 1.5 mL centrifuge tubes; 80 mM solution of the compound in DMSO was added in doses ranging from 0.125 to 5 mol equiv to hemin (pure DMSO was added to control samples). Then 100 μL of ultrapure water was added to each tube, and β-hematin formation initiated by the addition of 200.0 μL of 8 M acetate buffer (pH 5.2). The final concentration of DMSO per tube was kept constant at 25%. Tubes were incubated at 37.0 ± 0.1 °C for 18 h and then centrifuged. The remaining pellet was resuspended in 0.500 mL of DMSO to remove unreacted hematin. Tubes were then centrifuged again, DMSO-soluble fraction removed, and the pellet, consisting of a pure precipitate of β-hematin, dissolved in 1.000 mL of 0.1 M NaOH. Then 10.0 μL aliquots were transferred to 96-well microplate and diluted with 190.0 μL of 0.1 M NaOH (200.0 μL of 0.1 M NaOH was used as a blank). Absorbance was measured at 405 nm, with correction at 670 nm. A calibration curve of hemin dissolved in 0.1 M NaOH was made in the range $c_{HE} = (0.4–4.0) \times 10^{-5}$ M.

Acidity Constant (pK_a) Values Determination. Acidity constant were potentiometrically determined in MeOH:H_2O (1:1, v:v) at t = 25 ± 1 °C and at constant ionic strength (I = 0.1 M (NaCl)). Solutions of NaOH (0.1 M) and HCl (0.1 M) were prepared in MeOH:H_2O (1:1, v:v) and potentiometrically standardized. For working solution, studied compound was dissolved in methanol and diluted with the equivalent volume of aqueous 0.2 M NaCl ($c = (0.5–1.1) \times 10^{-3}$ M). Prior to titration, 100.0 μL of standard 0.1 M HCl solution was added to 4.00 mL of working compound solution. All probes were titrated with 2.0 μL increments of standard 0.1 M NaOH solution in 1.8–12.2 pH range. HyperQuad 2008 software[69] was used to calculate the value of acidity constant values (pK_{a1} and pK_{a2}) from four repeated titrations.

Inhibition Potential of Antiplasmodials 23 and 25 against the hERG K^+ Channel. Compounds **23** and **25** were submitted for a hERG K^+ channel inhibition test to evaluate the proarrhythmic potential that leads to Q–T prolongation cardiac toxicity. Extent of hERG channel inhibitory activity of the test compounds was assessed by Cyprotex US, LLC, Watertown, MA. A detailed protocol is available in the Supporting Information.

AMES Mutagenicity Screening of Antiplasmodial 23. Compound **23** was submitted to Cyprotex US, LLC, Watertown, MA for Ames mutagenicity screening and was found to be negative. A detailed protocol is available in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01374.

Relevant data and activity of synthesized antiplasmodials, tetraoxanes tested for activity in the liver stage, liver stage activity of tetraoxane antiplasmodials, BHIA plots, distribution diagrams, experimental procedures (PDF)

NMR spectra of synthesized compounds, HPLC analyses for purity (PDF)

Inhibition potential of antiplasmodials **23** and **25** against the hERG K^+ channel and AMES mutagenicity screening of antiplasmodial AMES mutagenicity screening of antiplasmodial AMES mutagenicity screening of anti-plasmodial 23 (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Ad, adamantane; ART, artemisinin; AQ, 4-amino-quinoline; CQ, chloroquine; MFQ, mefloquine; MLM, mouse liver microsomes; HLM, human liver microsomes; LS, liver stage; MOA, method of action; MAD, minimal active dose; MCD, minimal curative dose; MST, mean survival time; MTD, maximum tolerated dose; QPlogPow, predicted octanol/water partition coefficient; QPPMDCK, predicted apparent MDCK cell permeability for nonactive transport; QPlogHERG, predicted IC_{50} value for blockage of HERG K^+ channels

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