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In vitro efficiency of new acridyl derivatives against *Plasmodium falciparum*

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Abstract—A series of new 9-substituted acridyl derivatives were synthesized and their in vitro antimalarial activity was evaluated against one chloroquine-sensitive strain (3D7) and three chloroquine-resistant strains [W2 (Indochina), Bre1 (Brazil) and FCR3 (Gambia)] of *Plasmodium falciparum*. Some compounds inhibit the growth of malarial parasite with $IC_{50} \leqslant 0.20\ \mu M$.

1. Introduction

More than 40% of the population in the world lives in areas infected by malaria. Malaria is a disease that affects hundreds of millions of people, causes more than a million death per year, and is in continual increase.[1] Quinine (QN), then chloroquine (CQ) were the drugs of choice for malaria chemotherapy for over 50 years because of their high efficacy, relatively low toxicity, ease of use, and low cost.[2] However, in recent years, malaria parasites, particularly *Plasmodium falciparum*, the most virulent of the human parasite, have developed resistant strains to QN and CQ. Researches are in progress to elucidate the mechanism of action of those drugs that contain a quinoline nucleus and much effort is directed toward the synthesis of novel antimalarial drugs containing an acridine ring.[3–7]

Acridine compounds known to have antimalarial activity are essentially 9-aminoacridine derivatives such as quinacrine and 9-anilinoacridine derivatives.[7–11] It was previously considered that the primary receptor was DNA because acridines like chloroquine are able to intercalate into DNA and consequently can inhibit DNA transcription in parasites.[12–14] Furthermore, 9-anilinoacridines that have good antimalarial activities in vitro are potent inhibitors of parasite DNA topoisomerase II both in vitro and in situ.[15,16] This relationship is evidenced on 3,6-diamino-9-anilinoacridine that displays higher antiparasitic activity and increased inhibitory effects on parasite topoisomerase II. It was first suggested that low lipophilicity and high basicity were important factors for the in vitro antimalarial activity of 9-anilinoacridines.[7] Even if it appeared that there was no direct correlation between DNA binding and antimalarial activity, one can consider that the increased basicity and additional cationic charges resulting from the 3,6-diamino substitution of the acridine ring should greatly reinforce DNA binding. A model in which the acridine ring intercalates into DNA and the 9-anilino side group projects into the DNA minor groove where it interacts with topoisomerase II could explain the capacity of these compounds to inhibit the enzyme.[17]

However, it is recognized that inhibition of parasite topoisomerase II may not be the only mechanism of action of acridine derivatives. The antimalarial activity of

Abbreviations: β-Ala, β-alanine; Arg, arginine; Gly, glycine; Bn, benzyl; Boc, *tert*-butoxycarbonyle; Fmoc, fluorenyl-9-methoxycarbonyle; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyle; BOP, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DPPA, diphenylphosphoryl azide; TFA, trifluoroacetic acid.

Keywords: Antimalarial; *Plasmodium falciparum*; Aminoacridine; Peptidic synthesis.

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quinolines and acridine derivatives is actually believed to be due to the inhibition of hemoglobin digestion by the plasmodia, and subsequent blockade of the released free heme polymerization into hemozoin, the malaria pigment, in the parasite food vacuole.[18–21] The structure of hemozoin is thought to consist of a crystalline insoluble cyclic dimer of ferriprotoporphyrin IX.[22] Antimalarial drugs that contain an acridine nucleus can thus bind strongly to heme and inhibit the crystallization process.

As part of our research devoted to the synthesis of new DNA intercalators,[23] we have designed and synthesized new acridine derivatives, bearing one or several cationic charges and substituted at the 9-position by a chain of various length and nature. An in vitro investigation of the structure-antimalarial activity relationships will be reported.

2. Chemistry

Two series of acridine derivatives have been investigated. They are listed on Table 1. One is a series of 9-amino acridinium derivatives bearing several cationic charges in order to increase its pK_a: one on the acridine ring and the other(s) on the chain grafted at the 9-amino position. This chain can be a simple ammonium chain of six carbon atoms (compound **2**), or an amine-peptidic chain of various length and various nature. Aminoacids investigated in this work were Gly, β-Ala, alone or bound to Arg. Synthesis of these derivatives is described in Schemes 1 and 2. The second series of acridyl derivatives is positively charged only on the peptidic chain grafted on the acridine ring. Two derivatives (compounds **30** and **31**) have been synthesized. They differ by the connection of the peptidic chain to the acridine (NHCO vs CONH) and the total length of the chain. Synthesis of compound **30** is described in Scheme 3. Compound **31** was obtained by cleavage of the guanidinium protective group of the previously synthesized 9-acridinecarboxamide derivative.[23]

The general route of all the syntheses consists in a classical peptidic synthesis, using a series of peptidic couplings with coupling reagents to activate the acidic function. It requires the preliminary protection of the functional groups that are likely to react during the coupling. In order to avoid secondary coupling reactions, arginine used in this work was protected on its amine function. It was also protected on its guanidinium group, yielding derivatives easier to purify than the chloride salt and that protection was maintained until the ultimate stage of the synthesis. We chose to protect the guanidinium with the 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) protective group, as it is cleaved by TFA[24] while the arginine amine function was protected by the Fmoc-group, one of the very rare amine protective groups to be cleaved by bases.[25] Synthesis of the acridyl derivative bound to two β-Ala units and one arginine (compound **22)** was achieved by two parallel ways using one bis-protected β-Ala (O-Bn and N-Boc) and a bis-protected arginine as starting materials, on one hand, and acridine and one Boc-β-Ala moiety on the other hand (Scheme 2). This strategy allows obtaining more soluble synthons than the classical linear coupling method involved in the synthesis of the other amino acid derivatives. Yield of the final coupling and consequently, yield of compound **22** is thus increased. All the acridine derivatives were obtained as their TFA salts. As they were tested under their Cl^- form, the last step of the synthesis was a trifluoroacetate–chloride ion exchange through a Dowex Cl^- resin column, followed by precipitation of the product in H_2O/acetone to remove the sodium salts in excess.

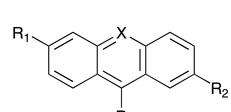
3. Antimalarial activity

The antimalarial activity of all the synthesized acridine derivatives has been tested on *P. falciparum* 3D7, W2, FCR3, and Bre1 strains (one chloroquine-sensitive and three chloroquine-resistant strains, respectively), and compared with that of chloroquine. Results are given in Table 2.

The data emphasize two major points:

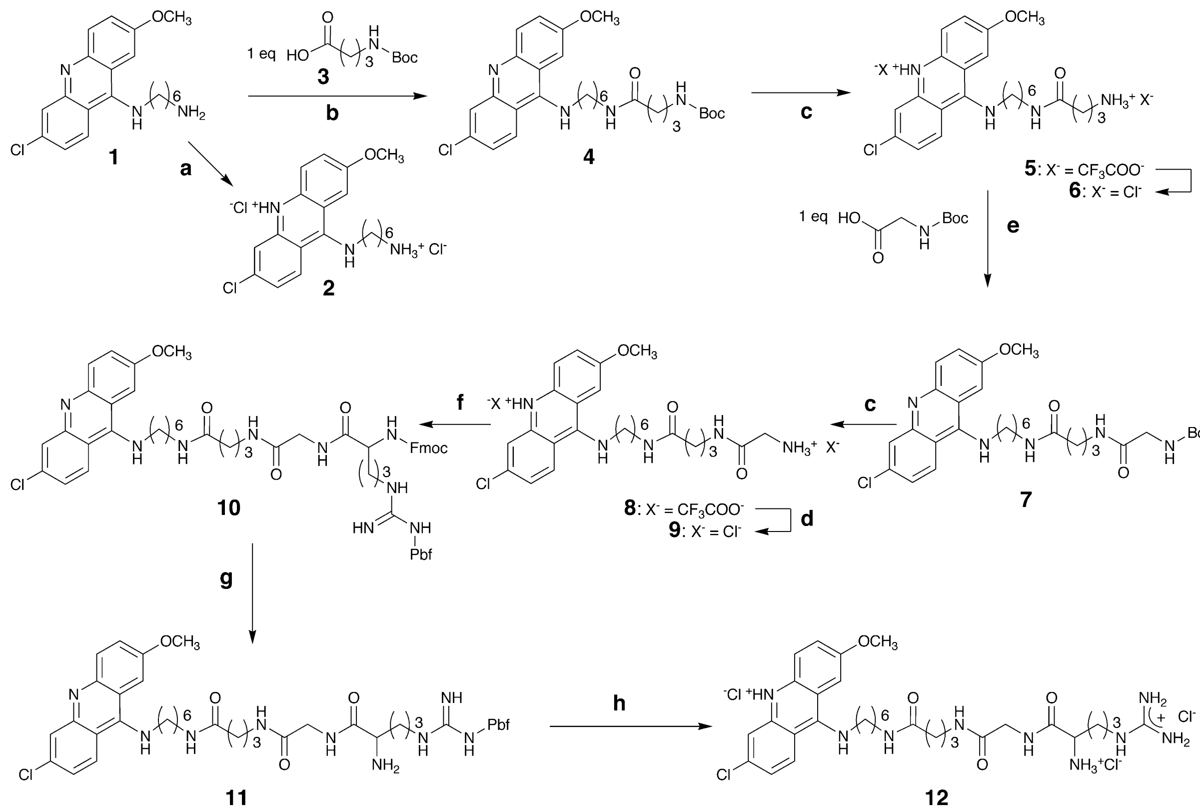
– Presence of a cationic charge on the acridine nucleus is required to provide a significant antimalarial activity. Compounds **30** and **31** in which the chain is not

Table 1. Acridine derivatives tested in this work



Compound	X	R_1	R_2	R
2	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NH_3{}^+Cl^-$
6	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NHCO(CH_2)_3NH_3{}^+Cl^-$
9	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NHCO(CH_2)_3NHCOCH_2NH_3{}^+Cl^-$
12	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NHCO(CH_2)_3NHCOCH_2NHArg^+Cl^-$
15	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NHCO(CH_2)_2NH_3{}^+Cl^-$
22	NH^+Cl^-	Cl	OCH_3	$NH(CH_2)_6NHCO(CH_2)_2NHCO(CH_2)_2NHArg^+Cl^-$
30	N	H	H	$NHCO(CH_2)_7NHCOCH_2NHArg^+Cl^-$
31	N	H	H	$CONH(CH_2)_6NHCOCH_2NHArg^+Cl^-$

Arg^+Cl^-=$COCH(NH_3{}^+Cl^-)(CH_2)_3NHC(=NH)(NH_3{}^+Cl^-)$.



Scheme 1. Synthesis of compounds **2**, **6**, **9**, and **12**. Reagents and conditions: (a) TFA, 12 h, Dowex Cl^-; (b) 1,1′-carbonyldiimidazole (1 equiv), CH_2Cl_2, rt, argon, 12 h; (c) CH_2Cl_2/TFA (1/1), rt, 12 h; (d) Dowex Cl^-; (e) 1,1′-carbonyldiimidazole (1.1 equiv), Et_3N (2 equiv/**5** + 1 equiv/Gly), DMF, rt, argon, 72 h; (f) (Fmoc)Arg(Pbf)OH (1.1 equiv), BOP (1.2 equiv), DIEA (5 equiv/**8** + 1.1 equiv/Arg), DMF, rt, argon, 72 h; (g) DBU (4 equiv), THF, rt, 2 h; (h) TFA/H_2O (95/5), Et_3SiH (2 equiv), rt, 12 h.

attached to the acridine by an amino bond, have $pK_a = 4$ and consequently are not positively charged on the ring in the in vitro assays. The IC_{50} values of their antimalarial activity range between 27–30 µM for **30** and 42–48 µM for **31**, according to the strain.

– The most active molecules are those whose chain attached to the acridine ring is shortest. Best results are obtained for the acridine derivative **2**, and IC_{50} values decrease in the order **9** > **12** > **6** > **2**, whatever the strains. However, the activity of **6** against Bre1 is almost equal to that of **2**, whereas it is about three times lower against the two other CQ-resistant strains.

In addition, compound **2** is between 2.5- and 3-fold more efficient than CQ against CQ-resistant strains.

The grafting of an arginine to compound **9** (compound **12**) hardly increases its activity. In contrast, it improves the activity against chloroquine-resistant strains of the series encompassing β-ala linkers (**22** > **15**), but severely decreases its activity against 3D7.

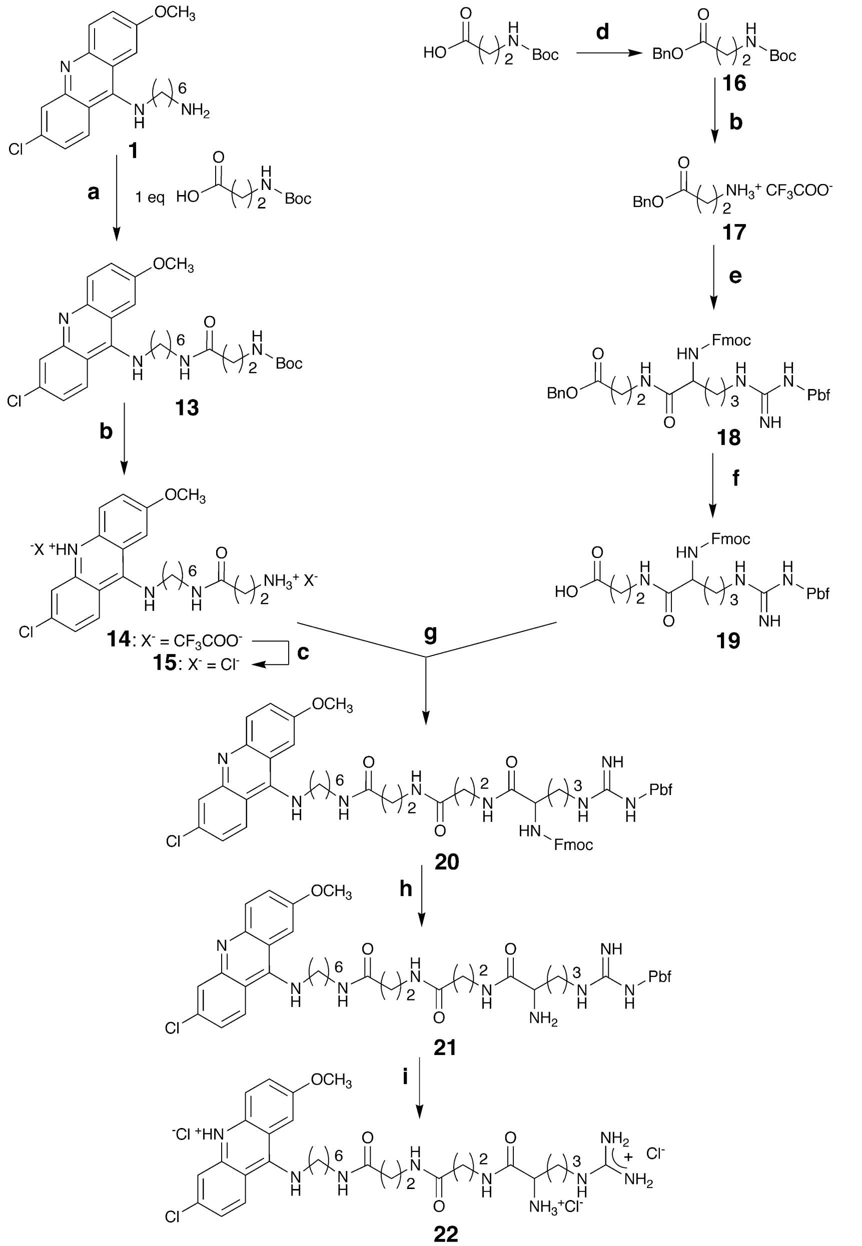
4. Discussion

It is obvious that the mechanism of action of antimalarial drugs is unclear and new data may help to better understand the role played by DNA or hematin.

Antimalarial 9-anilinoacridines are potent inhibitors of parasite DNA topoisomerase II both in vitro[15] and in situ.[16] Nevertheless, such as chloroquine, the 9-anilinoacridines can be considered inhibitors of heme polymerization or agents that act to divert heme from participating in the crystallization process, leading to the accumulation of free heme, which is toxic for the parasite.[26] Some of these compounds inhibit β-hematin formation, form drug–hematin complexes, and enhance hematin-induced lysis of erythrocytes. The nature of the substitution in the anilino ring affects these abilities. In addition, some 9-anilinoacridines show gametocytocidal activity.[27,28]

Compound **2**, the most active acridine derivative presented in this work, has a structure similar to that of mepacrine, a 9-aminoacridine synthetic anti-malarial drug. Its higher activity may result from its facility, due to its small size, to enter the vacuole.

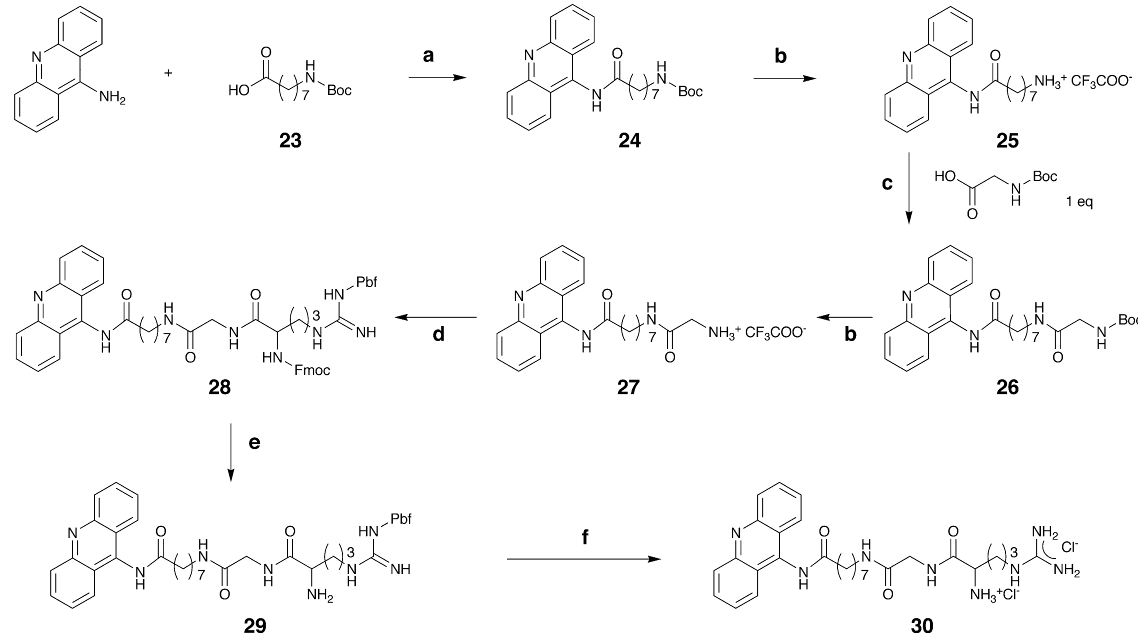
On the other hand, recent research has focused on acridine derivatives as chemotherapeutic agents because of the ability of the acridine chromophore to intercalate into DNA.[29] Based on this, the selection of 9-amino-6-chloro-2-methoxyacridine (ACMA) derivatives as intercalating agents has been widely developed.[30] As all the present synthesized acridine derivatives that reveal good inhibitors of *P. falciparum* are ACMA derivatives, it



Scheme 2. Synthesis of compounds **15** and **22**. Reagents and conditions: (a) 1,1′-carbonyldiimidazole (1 equiv), CH_2Cl_2, rt, argon, 12 h; (b) CH_2Cl_2/TFA (1/1), rt, 12 h; (c) Dowex Cl^-; (d) K_2CO_3 (1 equiv), DMF, BnBr (1 equiv), rt; (e) (Fmoc)Arg(Pbf)OH (0.83 equiv), DPPA (1 equiv), DMF, DIEA (1.33 equiv), 0 °C, 12 h; (f) H_2 (10 bars), Pd/C, EtOAc, 48 h; (g) BOP (1 equiv), DIEA (2 equiv/**14** + 2 equiv/**19**), DMF, rt, argon, 28 h; (h) DBU (1 equiv), THF, rt, 72 h; (i) TFA/H_2O (90/10), Et_3SiH (2 equiv), rt, 12 h.

seems reasonable to consider that our molecules are also good DNA intercalators. The positive charge on the acridine ring favors the approach of the molecule to DNA and reinforces DNA-interaction. This is supported by fluorescence experiments, that have demonstrated that compound **22** is able to interact strongly with poly(dAdT)$_2$ and poly(dGdC)$_2$, while compound **30** displays no detectable interaction with DNA.[31] The positively charged acridine side chain may lie in the minor groove and interacts with DNA phosphate groups. If it contains an arginine moiety (compounds **12** and **22**), it projects rather in the major groove and the amino groups of the guanidinium side chain of arginine can form hydrogen bonds with guanines.[32] So, the antimalarial activity of 9-aminoacridine derivatives could be related to their ability to intercalate into DNA.

In addition, the planarity of acridine nucleus favors the formation of cofacial π–π complexes with the free released heme. A NMR study reported that the interaction between CQ and hematin consists of a close stacking between the porphyrin ring and the quinoline nucleus of the drug.[33] The complexes may also be stabilized by hydrogen bonding between the protonated terminal amino groups of the acridine side chain and the propionate carboxylate of the porphyrin. As these interactions are hydrophobic and can occur between two neutral rings, a positive charge on the acridine ring would not



Scheme 3. Synthesis of compound **30**. Reagents and conditions: (a) BOP (1.1 equiv), DIEA (1.2 equiv), DMF, rt, argon, 12 h; (b) CH_2Cl_2/TFA (1/1), rt, 12 h; (c) BOP (1.2 equiv), DIEA (5 equiv/**25** + 1.2 equiv/Gly), DMF, rt, argon, 12 h; (d) (Fmoc)Arg(Pbf)OH (1 equiv), BOP (1.2 equiv), DIEA (5 equiv/**27** + 1.2 equiv/Arg), DMF, rt, argon, 12 h; (e) DBU (4 equiv), THF, rt, 2 h; (f) TFA/H_2O (95/5), Et_3SiH (2 equiv), rt, 12 h.

Table 2. Comparative in vitro efficiency of chloroquine (CQ) and the acridine derivatives against chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains

Plasmodium falciparum strains							
Chloroquine-sensitive		Chloroquine-resistant					
3D7		W2		FCR3		Bre1	
Compound	IC_{50} (μM) [confidence interval]	Compound	IC_{50} (μM) [confidence interval]	Compound	IC_{50} (μM) [confidence interval]	Compound	IC_{50} (μM) [confidence interval]
CQ	0.018 [0.016–0.020]	**2**	0.18 [0.13–0.25]	**2**	0.20 [0.18–0.23]	**2**	0.17 [0.13–0.21]
2	0.13 [0.09–0.19]	CQ	0.44 [0.38–0.51]	CQ	0.50 [0.39–0.64]	**6**	0.18 [0.12–0.19]
6	0.19 [0.14–0.27]	**6**	0.57 [0.47–0.68]	**6**	0.54 [0.50–0.59]	**12**	0.50 [0.42–0.58]
12	0.84 [0.76–0.93]	**12**	0.82 [0.73–0.91]	**12**	0.67 [0.63–0.71]	CQ	0.52 [0.42–0.66]
9	0.89 [0.75–1.05]	**9**	0.83 [0.69–1.01]	**9**	0.81 [0.65–1.01]	**9**	0.62 [0.53–0.72]
15	0.91 [0.71–1.16]	**22**	0.94 [0.79–1.13]	**22**	0.86 [0.77–0.95]	**22**	0.86 [0.79–0.92]
22	3.32 [3.07–3.58]	**15**	1.25 [0.97–1.61]	**15**	1.25 [0.98–1.60]	**15**	1.26 [1.13–1.41]
30	27.1 [24.5–30.0]	**30**	30.5 [26.9–34.7]	**30**	27.2 [23.0–32.2]	**30**	28.7 [24.5–33.6]
31	42.0 [38.7–45.5]	**31**	43.6 [36.7–51.7]	**31**	42.5 [32.3–56.0]	**31**	47.6 [42.8–53.1]

strengthen the stacking and improve the activity. Even if antimalarial activity of our acridine derivatives should result from the strength of their binding to heme, it must also depend strongly on their ability to accumulate in the food vacuole of the parasite. Fairly high basicity as observed here for the active compounds ($pK_a > 8$) facilitates the accumulation in the malaria parasite acidic food vacuole in which hemozoin formation takes place.[34]

The role played both by the acridine chromophore and the basicity of the molecule is highlighted by the fact that most of the present compounds were found to be more potent against *P. falciparum* parasites than arylsulfonyl acridinyl derivatives.[5] The antimalarial activity of these sulfone compounds has been tested on *P. falciparum* 3D7 and W2 strains, and it has been concluded that, even if the sulfonyl group, in increasing the basicity of the molecule, was essential for the antimalarial activity, the action was conferred by the acridine ring.

In conclusion, some of the acridine derivatives evaluated in this study showed significant antimalarial activity against *P. falciparum* in vitro. This activity is conferred by the acridine ring, in its cationic form, and is dependent on the nature of the chain linked to the ring. So, all the mechanisms postulated to elucidate the antimalarial activity of drugs that contain a quinoline ring could be discussed in this work and research is in progress to optimize the structure of these derivatives. Subsequent experiments will be performed in the laboratory in order to find a correlation between the activity of our compounds and their ability to bind to DNA and/or to form complexes with ferriprotoporphyrin IX.

5. Experimental

5.1. Chemistry

5.1.1. General methods. Coupling reactions were performed under an argon atmosphere and in a commercial acid- and base-free DMF (99.8% Aldrich). Most of the reactions were carried out in the dark. THF was dried over sodium and distilled prior to use. All other solvents and reagents were pure grade and used without purification, as well as the following commercially available chemicals: 9-aminoacridine and 8-aminocaprylic acid (Acros), 6,9-dichloro-2-methoxyacridine and 1,6-hexanediamine (Aldrich), γ-aminobutyric acid, Boc-Gly-OH, and Boc-β-Ala-OH (Fluka), and Fmoc-Arg(Pbf)-OH (Senn Chemicals).

Reactions were monitored by thin layer chromatography (TLC) performed on silica gel sheets containing UV fluorescent indicator (60 F254 Merck). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 200, Bruker AC 250, Bruker AV 360, and Bruker AC 400 spectrometers (200, 250, 360, and 400 MHz for ^{1}H, respectively, and 50, 63, 90, and 100 MHz for ^{13}C, respectively). Chemical shifts, δ, are reported in ppm taking residual $CHCl_3$ or CHD_2OD as the reference. Mass spectra were recorded on a Finnigan-MAT-95-S, using $MeOH/CH_2Cl_2/H_2O$ (45/40/15, v/v) as solvent. Elemental analyses were performed by the Service Central de Microanalyse du CNRS.

5.1.2. *N*^{1}-(6-Chloro-2-methoxyacridin-9-yl)hexane-1,6-diamine (1). This compound was synthesized from 6,9-dichloro-2-methoxyacridine (1.1 g, 2.98 mmol) and 1,6-hexanediamine (14 mL), according to the procedure described by Uno[35], and obtained in 98% yield.

- ^{1}H NMR (CD_3OD, 200 MHz) δ ppm: 1.20 (m, 6H), 1.55 (qn, 2H), 2.40 (t, 2H), 3.55 (t, 2H), 3.80 (s, 3H), 7.05 (dd, 1H), 7.20 (dd, 1H), 7.25 (s, 1H), 7.60 (d, 1H), 7.65 (s, 1H), 8.00 (d, 1H).
- ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 27.6, 27.8, 32.1, 33.6, 42.3, 50.9, 56.1, 101.0, 115.6, 118.2, 124.0, 125.9, 126.9, 130.3, 130.5, 136.3, 146.9, 149.2, 152.7, 157.0.
- MS (ES) *m/z*: 358.2 (MH^+) (100%).
- Anal. Calcd for $C_{20}H_{24}ClN_3O$, H_2O: C, 63.91; H, 6.97; N, 11.18. Found C, 64.02; H, 6.78; N, 11.02.

5.1.3. 4-(*tert*-Butoxycarbonylamino)butyric acid (3). Di-*tert*-butyl dicarbonate [$(Boc)_2O$] (3.27 g, 15 mmol) in 15 mL dioxane was added dropwise to a solution of γ-aminobutyric acid (1.03 g, 10 mmol) in dioxane (15 mL) and NaOH 1 N (15 mL) at 0 °C. The reaction mixture was allowed to stand overnight. The crude product was concentrated under reduced pressure, HCl 1 N was added, and diluted with EtOAc. The organic layer was washed with water and dried over Na_2SO_4. The solvant was evaporated in vacuo. The compound **3** was obtained as a yellow oil in 85% yield (1.83 g, 9 mmol) and used without further purification.

- ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.35 (s, 9H), 1.75 (qn, 2H), 2.35 (t, 2H), 3.10 (q, 2H), 4.85 (t, 1H), 6.05 (s, 1H).
- ^{13}C NMR ($CDCl_3$, 63 MHz) δ ppm: 24.8, 28.0, 30.9, 39.5, 78.9, 156.1, 177.2.
- MS (ES) *m/z*: 202.2 (MH^+) (100%).

5.1.4. *tert*-Butyl 4-[6-(6-chloro-2-methoxyacridin-9-ylamino)hexylamino]-4-oxobutylcarbamate (4). A mixture of compound **3** (788 mg, 3.88 mmol) and 1,1′-carbonyldiimidazole (629 mg, 3.88 mmol) in CH_2Cl_2 (20 mL) was stirred for 1 h. A solution of **1** (1.39 g, 3.88 mmol) in CH_2Cl_2 (20 mL) was added dropwise and the reaction mixture was stirred overnight, then concentrated in vacuo and purified by MPLC in CH_2Cl_2/MeOH (90/10) to afford **4** as a yellow powder in 55% yield (1.16 g, 2.14 mmol).

- ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm: 1.30 (m, 15H), 1.65 (m, J = 8 Hz, 4H), 2.15 (t, J = 8 Hz, 2H), 3.10 (m, 4H), 3.55 (t, J = 8 Hz, 2H), 3.90 (s, 3H), 4.90 (m, 1H), 6.45 (m, 1H), 7.15 (dd, J = 2.2 Hz, J_2 = 9.7 Hz, 2H), 7.18 (dd, J_1 = 2.2 Hz, J_2 = 9.7 Hz, 1H), 7.35 (dd, J_1 = 2.2 Hz, J_2 = 9.7 Hz, 1H), 7.85 (s, 1H), 7.92 (dd, J_1 = 2.2 Hz, J_2 = 9.7 Hz, 2H), 7.95 (s, 1H).
- ^{13}C NMR ($CDCl_3$, 50 MHz) δ ppm: 26.2, 26.5, 28.3, 29.3, 31.4, 33.5, 39.0, 39.5, 50.2, 55.4, 65.0, 79.0, 99.2, 115.5, 117.7, 124.1, 124.2, 124.4, 127.8, 131.1, 134.6, 146.5, 148.2, 149.6, 149.8, 155.8, 172.6.
- MS (ES) *m/z*: 543.2 (MH^+) (100%).

5.1.5. 9-[6-(4-Ammoniobutanamido)hexylamino]-6-chloro-2-methoxyacridinium ditrifluoroacetate (5). Acridine **4** (1.16 g, 2.14 mmol) was dissolved in TFA/CH_2Cl_2 (1/1) (13 mL). After stirring overnight in the dark, the solvent was evaporated in vacuo. The TFA salt **5** was obtained quantitatively and used without further purification.

- ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.50 (m, 6H), 1.98 (m, 4H), 2.35 (t, J = 6.2 Hz, 2H), 3.16 (t, J = 6.2 Hz, 2H), 3.30 (t, J = 6.2 Hz, 2H), 3.32 (t, J = 6.2 Hz, 2H), 3.99 (s, 3H), 4.11 (t, J = 7.2 Hz, 2H), 7.46 (dd, J_1 = 2.5 Hz, J_2 = 7.2 Hz), 7.48 (dd, J_1 = 2.5 Hz, J_2 = 7.2 Hz, 1H), 7.60 (dd, J_1 = 2.5 Hz, J_2 = 7.2 Hz, 1H), 7.65 (dd, J_1 = 2.5 Hz, J_2 = 7.2 Hz, 1H), 7.70 (dd, J_1 = 2.5 Hz, J_1 = 7.2 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H).
- ^{13}C NMR (CD_3OD, 63 MHz) δ ppm: 22.7, 25.8, 28.3, 29.1, 32.3, 38.7, 48.7, 55.3, 62.4, 117.3, 119.9, 123.6, 127.1, 140.4, 152.0, 156.0, 172.6.
- MS (ES) *m/z*: 443.2 (MH^+) (100%). MS (HRMS) *m/z* calcd for $C_{24}H_{32}ClN_4O_2$ (MH^+): 443.2208; found: 443.2209.

5.1.6. *tert*-Butyl 2-{4-[6-(6-chloro-2-methoxyacridin-9-ylamino)hexylamino]-4-oxobutylamino}-2-oxoethylcarbamate (7). A mixture of Boc-Gly-OH (376 mg, 2.14 mmol), BOP (1,14 g, 2.58 mmol), and DIEA (450 μL, 2.58 mmol) in DMF (11 mL) was stirred for 15 min to allow the formation of the activated ester. Compound **5** (946 mg, 2.14 mmol) and DIEA (1.87 mL, 10.7 mmol) were dissolved in DMF (11 mL) and added dropwise to the activated ester. The reaction mixture was stirred overnight in the dark at room temperature. DMF was removed under reduced pressure and the residue was dissolved in acetone (14 mL) and added dropwise to a stirred solution of 5% $NaHCO_3$ (140 mL), according to the procedure previously developed by Kossanyi et al.[36] The mixture was allowed to stand for 24 h at room temperature. The resulting precipitate was filtered and dried, and acridine **7** was obtained as an amorphous powder in 73% yield (940 mg, 1.57 mmol), after MPLC in CH_2Cl_2/MeOH (90/10).

– ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.41 (m, 15H), 1.80 (m, 4H), 2.18 (t, J = 5.7 Hz, 2H), 3.25 (t, J = 5.7 Hz, 2H), 3.30 (t, J = 5.7 Hz, 2H), 3.75 (m, 4H), 3.87 (s, 3H), 5.40 (m, 1H), 6.50 (m, 1H), 6.61 (m, 1H), 7.08 (d, J = 2.4 Hz, 1H), 7.16 (dd, J_1 = 2.4 Hz, J_2 = 9.3 Hz), 7.20 (d, J = 2.4 Hz, 1H), 7.28 (dd, J_1 = 2.4 Hz, J_2 = 9.3 Hz, 1H), 7.73 (d, J = 9.3 Hz, 1H), 7.83 (s, 1H), 7.96 (d, J = 9.3 Hz, 1H).
– ^{13}C NMR (CD_3OD, 63 MHz) δ ppm: 26.7, 27.4, 28.6, 30.2, 30.6, 34.2, 39.6, 40.1, 44.7, 50.1, 56.6, 64.3, 80.6, 103.5, 110.8, 115.0, 118.6, 121.0, 121.4, 124.8, 128.3, 128.9, 135.5, 141.1, 141.4, 152.7, 157.8, 158.3, 175.2.
– MS (ES) m/z: 600.3 (MH^+) (100%).

5.1.7. 9-{6-[4-(2-Ammonioacetamido)butanamido]hexylamino}-6-chloro-2-methoxyacridinium ditrifluoroacetate (8). Dissolution of acridine **7** (940 mg, 1.57 mmol) in TFA/CH_2Cl_2 (1/1) (5 mL) followed by the same treatment as applied to **4** afforded the crude TFA salt **8**, which was used in the next step without further purification.

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.50 (m, 6H), 1.79 (qn, J = 6.8 Hz, 2H), 1.98 (qn, 2H), 2.21 (t, J = 6.8 Hz, 2H), 3.18 (t, J = 6.8 Hz, 2H), 3.26 (t, J = 6.8 Hz, 2H), 3.65 (s, 2H), 3.99 (s, 3H), 4.12 (t, J = 7.2 Hz, 2H), 7.50 (dd, J_1 = 2.0 Hz, J_2 = 7.9 Hz, 1H), 7.60 (dd, J_1 = 2.0 Hz, J_2 = 7.9 Hz, 1H), 7.70 (dd, J_1 = 2.0 Hz, J_2 = 7.9 Hz, 1H), 7.80 (dd, J_1 = 2.0 Hz, J_2 = 7.9 Hz, 2H), 8.44 (d, J = 9.3 Hz, 1H).
– ^{13}C NMR (CD_3OD, 63 MHz) δ ppm: 22.7, 25.8, 28.3, 29.1, 32.3, 38.7, 41.4, 48.7, 55.3, 62.4, 103.0, 117.3, 119.9, 123.6, 127.1, 140.4, 152.0, 156.0, 172.0.
– MS (ES) m/z: 500.2 (MH^+) (100%). MS (HRMS): m/z calcd for $C_{26}H_{35}ClN_5O_3$ (MH^+): 500.2423; found: 500.2429.

5.1.8. (9*H*-Fluoren-9-yl)methyl 22-(6-chloro-2-methoxyacridin-9-ylamino)-1-imino-7,10,15-trioxo-1-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamido)-2,8,11,16-tetraazadocosan-6-ylcarbamate (10). A mixture of Fmoc-Arg (Pbf)-OH (1.12 g, 1.73 mmol), BOP (0.84 g, 1.90 mmol), and DIEA (330 µL, 1.90 mmol) in DMF (10 mL) was stirred at room temperature for 30 min. Acridine **8** (1.57 mmol) was dissolved in DMF (10 mL) with DIEA (1.36 mL, 7.85 mmol) and added dropwise to the activated ester. The reaction mixture was stirred for 3 days, then the solvent was evaporated. Acetone (10 mL) was added to dissolve the crude residue and the solution was poured dropwise into a solution of 5% $NaHCO_3$ (100 mL). The mixture was allowed to stand overnight and the resulting precipitate was filtered, dried, and purified by MPLC using gradient of CH_2Cl_2/MeOH from 2% to 15%. Acridine **10** was obtained in 58% yield (1.03 g, 0.91 mmol).

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.10–1.40 (m, 12H), 1.40 (m, 2H), 1.65 (m, 6H), 1.98 (s, 3H), 2.03 (t, 2H), 2.38 (s, 3H), 2.45 (s, 3H), 2.80 (s, 2H), 2.95 (t, 2H), 3.10 (m, 4H), 3.65 (t, 2H), 3.78 (m, 2H), 3.84 (s, 3H), 3.90 (t, 1H), 4.00 (q, 1H), 4.25 (t, 2H), 7.05–7.25 (m, 5H), 7.30 (dd, J_1 = 2.2 Hz, J_2 = 9.7 Hz, 1H), 7.40–7.50 (m, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.70 (dd, J_1 = 9.7 Hz, 2H), 8.13 (d, J = 9.7 Hz, 1H).
– ^{13}C NMR (CD_3OD, 63 MHz) δ ppm: 12.5, 18.4, 19.6, 26.7, 27.5, 28.6, 30.2, 31.0, 32.0, 34.2, 39.7, 40.0, 43.9, 44.0, 46.2, 56.4, 59.9, 63.5, 69.7, 87.6, 102.2, 109.4, 120.9, 124.4, 126.1, 126.7, 127.9, 128.1, 128.8, 133.4, 134.3, 139.0, 142.3, 145.0, 148.3, 151.6, 157.5, 158.0, 158.7, 171.5, 172.5, 175.0.
– MS (ES) m/z: 1130.3 (MH^+) (100%).

5.1.9. 2-Amino-*N*-(2-{4-[6-(6-chloro-2-methoxyacridin-9-ylamino)hexylamino]-4-oxobutylamino}-2-oxoethyl)-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidine]-pentanamide (11). DBU (340 µL, 2.28 mmol) was added to a solution of acridine **10** (644 mg, 0.57 mmol) in THF (5 mL) and the solution was stirred at room temperature for 2 h to drive the reaction to completion. The reaction mixture was poured into diethyl ether (100 mL) whilst stirring. The precipitate was filtered, washed with diethyl ether, and dried. It was dissolved in CH_2Cl_2 (50 mL) and the solution was washed with water (3 × 50 mL), then brine, and dried over Na_2SO_4. After evaporation in vacuo, acridine **11** was obtained in quantitative yield and used in the next step without further purification.

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.30 (m, 6H), 1.39 (s, 6H), 1.55 (m, 2H), 1.75 (m, 6H), 2.00 (s, 3H), 2.15 (t, J = 7.5 Hz, 2H), 2.46 (s, 3H), 2.54 (s, 3H), 2.91 (s, 2H), 3.15 (m, 6H), 3.80 (m, 4H), 3.93 (s, 3H), 7.24 (dd, J_1 = 2.5 Hz, J_2 = 9.3 Hz, 1H), 7.38 (dd, J_1 = 2.5 Hz, J_2 = 9.3 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.82 (dd, J_1 = 2.2 Hz, J_2 = 9.3 Hz, 2H), 7.85 (s, 1H), 8.22 (d, J = 9.3 Hz, 1H).
– ^{13}C NMR (CD_3OD, 90 MHz) δ ppm: 12.5, 14.5, 18.4, 19.6, 20.4, 20.9, 24.9, 26.7, 27.4, 27.6, 28.7, 30.3, 32.0, 33.1, 34.1, 39.3, 39.8, 40.2, 43.4, 43.9, 50.3, 50.9, 55.3, 55.7, 56.6, 87.6, 101.2, 115.6, 118.4, 124.1, 126.0, 126.1, 126.8, 127.2, 130.2, 133.4, 136.4, 139.3, 147.0, 149.3, 152.9, 157.2, 158.0, 159.8, 171.5, 175.3, 178.2.
– MS (ES) m/z: 454.6 ($M+2H^+$)/2 (100%), 908.3 (MH^+) (45%).

5.1.10. 9-(1-Amino-6-ammonio-1-iminio-7,10,15-trioxo-2,8,11,16-tetraazadocosan-22-ylamino)-6-chloro-2-methoxyacridinium trichloride (12). Acridine **11** (250 mg, 0.275 mmol) was dissolved in TFA/H_2O (95/5) (25 mL), triethylsilane was added (88 µL, 0.550 mmol), and the resulting solution was stirred at room temperature overnight. The solvents were removed under reduced pressure. The crude residue was dissolved in water (1 mL) and this solution was passed through a column of ion exchange resin Dowex Cl^-. The aqueous layer was lyophilized and the product was precipitated in H_2O/acetone to afford **12** as a yellow powder in 48% yield (87 mg, 0.132 mmol).

- ^{1}H NMR (CD_3OD, 400 MHz) δ ppm: 1.46 (q, $J = 6.7$ Hz, 2H), 1.54 (q, $J = 6.7$ Hz, 4H), 1.81 (m, 2H), 2.06 (q, $J = 7.3$ Hz, 4H), 2.26 (s, 2H), 3.20 (t, 2H), 3.21 (m, 2H), 3.26 (m, 2H), 3.78 (m, 2H), 3.96 (s, 2H), 4.03 (s, 3H), 4.10 (t, 1H), 4.19 (m, 2H), 7.50 (dd, $J_1 = 2.2$ Hz, $J_2 = 9.5$ Hz, 1H), 7.65 (dd, $J_1 = 2.2$ Hz, $J_2 = 9.5$ Hz, 1H), 7.82 (dd, $J_1 = 2.2$ Hz, $J_2 = 9.5$ Hz, 2H), 7.85 (s,1H), 8.50 (d, $J = 9.5$ Hz, 1H).
- ^{13}C NMR (CD_3OD, 100 MHz) δ ppm: 19.0, 23.5, 23.9, 25.3, 26.1, 28.0, 28.6, 28.7, 29.2, 37.9, 38.5, 38.9, 40.4, 42.1, 47.0, 47.2, 47.4, 47.6, 47.8, 48.1, 48.3, 48.9, 52.8, 55.6, 59.6, 60.9, 67.2, 102.9, 117.1, 120.1, 123.7, 127.4, 140.4, 156.6, 156.8, 157.2, 166.1, 169.2, 169.8, 174.2, 177.7, 180.0.
- UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 280 (55,560), 340 (5060), 422 (10,300), 439 (9560).
- MS (ES) *m*/*z*: 328.7 ($M+2H^+$)/2 (100%), 656.4 (MH^+) (35%).
- Anal. Calcd for $C_{32}H_{49}Cl_4N_9O_4$, NaCl, $2H_2O$: C, 44.69; H, 6.21; N, 14.66. Found C, 44.68; H, 6.11; N, 14.18.

5.1.11. *tert*-Butyl 3-[6-(6-chloro-2-methoxyacridin-9-yl-amino)hexylamino]-3-oxopropylcarbamate (13). Synthesis of acridine **13** was performed by coupling of **1** and Boc-β-Ala-OH, using the procedure already applied to the synthesis of **4**. Acridine **13** was obtained in 54% yield, after purification by chromatography using CH_2Cl_2/MeOH (95/5).

- ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm: 1.37 (m, 15H), 1.66 (qn, $J = 5.8$ Hz, 2H), 2.30 (t, $J = 6$ Hz, 2H), 3.22 (q, $J = 6.3$ Hz, 2H), 3.31 (q, $J = 6$ Hz, 2H), 3.60 (t, $J = 6.9$ Hz, 2H), 3.88 (s, 3H), 5.31 (t, 1H), 6.19 (t, 1H), 7.20 (m, 2H), 7.34 (dd, $J_1 = 2.4$ Hz, $J_2 = 9.3$ Hz, 1H), 7.90 (m, 2H), 7.94 (d, $J = 9.5$ Hz, 1H).
- ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 26.2, 28.3, 29.3, 31.3, 36.3, 36.7, 38.9, 50.1, 55.5, 79.3, 99.4, 115.3, 117.6, 124.2, 124.3, 124.5, 127.4, 130.6, 134.9, 146.0, 147.0, 150.0, 155.8, 171.3.
- MS (ES) *m*/*z*: 529.2 (MH^+) (100%).

5.1.12. 9-[6-(3-Ammoniopropanamido)hexylamino]-6-chloro-2-methoxyacridinium ditrifluoroacetate (14). The TFA salt **14** was obtained by the same treatment as applied to **4** and was used in the next step without further purification.

- ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.40–1.58 (m, 8H), 1.97 (t, 2H), 3.22 (m, 2H), 3.92 (s, 3H), 4.01 (t, 2H), 7.34 (d, $J = 9.3$ Hz, 1H), 7.45 (d, $J = 9.5$ Hz, 1H), 7.56 (m, 2H), 8.24 (d, $J = 9.5$ Hz, 2H).
- ^{13}C NMR (CD_3OD, 90 MHz) δ ppm: 26.2, 28.8, 29.3, 31.4, 35.7, 38.8, 49.0, 55.3, 114.0, 117.2, 120.1, 123.6, 127.3, 130.0, 132.0, 141.0, 159.0, 171.0.
- MS (ES) *m*/*z*: 429.2 (MH^+) (100%). MS (HRMS): *m*/*z* calcd for $C_{23}H_{30}ClN_4O_2$ (MH^+): 429.2052; found: 429.2065.

5.1.13. Benzyl 3-(*tert*-butoxycarbonylamino)propionate (16). Boc-β-Ala-OH (8 g, 42.3 mmol) was dissolved in DMF (20 mL). K_2CO_3 (5.85 g, 42.3 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then a solution of BnBr (5.03 mL, 42.3 mmol) in DMF (10 mL) was added dropwise at room temperature. The reaction mixture was stirred overnight. After filtration and evaporation, the crude product was purified by chromatography over silica gel with pentane/diethyl ether (90/10) to afford **16** in 70% yield (8.261 g, 29.6 mmol).

- ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.40–1.55 (m, 9H), 2.61 (t, $J = 7.2$ Hz, 2H), 3.46 (q, $J = 7.2$ Hz, 2H), 5.24 (s, 2H), 7.39 (s, 5H).
- ^{13}C NMR ($CDCl_3$, 90 MHz) ppm: 28.2, 34.5, 36.0, 66.2, 79.1, 128.0, 128.1, 128.4, 135.5, 155.6, 172.1.
- MS (ES) *m*/*z*: 302.1 (MNa^+) (100%).

5.1.14. 2-Benzyloxycarbonyl-ethyl-ammonium trifluoroacetate (17). The TFA salt **17** was obtained by the same treatment as applied to **4** and was used in the next step without further purification.

- ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 2.81 (m, 2H), 3.30 (m, 2H), 5.15 (s, 2H), 7.37 (s, 5H), 7.93 (m, 3H).
- ^{13}C NMR ($CDCl_3$, 90 MHz) δ ppm: 30.6, 35.7, 67.3, 128.2, 128.5, 128.6, 134.8, 171.6.

5.1.15. Benzyl 3-(2-{[(9*H*-fluoren-9-yl)methoxy]carbonylamino}-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino]pentanamido)propionate (18). DPPA (4.07 mL, 18.76 mmol) was added to a solution of Fmoc-Arg-(Pbf)-OH (10.14 g, 15.63 mmol) in DMF (250 mL). The mixture was stirred for 1 h at 0 °C. Then a solution of **17** (5.25 g, 18.76 mmol) in DMF (100 mL) and DIEA (4.35 mL, 25 mmol) was added dropwise at 0 °C. The reaction mixture was stirred overnight. After evaporation of the solvent and chromatography with heptane/EtOAc (90/10), **18** was obtained in 81% yield (relative to arginine) (10.2 g, 12.61 mmol).

- ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.37 (s, 6H), 1.50 (m, 2H), 1.70 (m, 2H), 2.01 (s, 3H), 2.45 (s, 3H), 2.53 (s, 3H), 2.85 (s, 2H), 3.19 (m,2H), 3.47 (m, 2H), 4.10 (m, 2H), 4.29 (d, $J = 7.3$ Hz), 5.00 (s, 2H), 5.95, (m, 1H), 6.20 (m, 1H), 7.28 (m, 9H), 7.51 (d, $J = 7.6$ Hz, 2H), 7.68 (d, $J = 7.6$ Hz, 2H).
- ^{13}C NMR ($CDCl_3$, 63 MHz) δ ppm: 12.4, 19.2, 25.2, 28.4, 46.9, 53.3, 86.3, 91.0, 108.2, 117.5, 119.8, 124.6, 127.0, 127.6, 128.1, 128.2, 128.4, 132.1, 135.6, 138.2, 141.1, 143.6, 156.4, 158.7, 172.0.
- MS (ES) *m*/*z*: 832.5 (MNa^+) (100%), 810.5 (MH^+) (32%).

5.1.16. 3-(2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl-sulfonyl)guanidine]pentanamido)propionic acid (19). One gram of Pd/C was added to a solution of benzylester derivative **18** (10.2 g, 12.6 mmol) in EtOAc (200 mL). Compound **19** was obtained by hydrogenolysis after 48 h under pressure (10 bars). Filtration over Celite and evaporation afforded a white solid, which was crystallized in heptane/EtOAc. Compound **19** was obtained in 94% yield (8.56 g, 11.9 mmol).

- ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.17 (m, 6H), 1.42 (m, 2H), 1.45 (m, 2H), 1.94 (s, 3H), 2.37–2.46 (m, 8H), 2.80 (s, 2H), 3.15 (m, 2H), 3.43 (m, 1H), 4.00 (t, 1H), 4.17 (m, 2H), 6.41 (m, 2H), 7.20 (m, 4H), 7.56 (d, J = 10.4 Hz, 2H), 7.63 (d, J = 10.4 Hz, 2H).
- ^{13}C NMR ($CDCl_3$, 63 MHz) δ ppm: 12.4, 17.9, 19.2, 25.1, 28.4, 29.6, 34.0, 35.4, 40.2, 43.0, 46.8, 50.4, 54.5, 67.0, 86.4, 117.5, 119.8, 123.9, 124.7, 125.1, 126.8, 127.0, 127.6, 132.2, 138.3, 141.1, 143.5, 143.7, 156.6, 158.8, 173.0, 175.5.
- MS (ES) m/z: 720.3 (MH^+) (100%).

5.1.17. (9*H*-Fluoren-9-yl)methyl 22-(6-chloro-2-methoxyacridin-9-ylamino)-1-imino-7,11,15-trioxo-1-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamido)-2,8,12,16-tetraazadocosan-6-ylcarbamate (20). DIEA (393 µL, 2.26 mmol) was added to a solution of **19** (0.81 g, 1.13 mmol) in DMF (10 mL) and BOP (0.5 g, 1.13 mmol). The resulting solution was stirred for 30 min, and a solution of acridine derivative **14** (1.13 mmol) and DIEA (393 µL, 2.26 mmol) in DMF (20 mL) was added dropwise. The reaction mixture was stirred for 48 h at room temperature. The DMF was evaporated, the residue was dissolved in acetone (10 mL) and added dropwise to a solution of 5% $NaHCO_3$ (100 mL). The mixture was allowed to stand for 24 h at room temperature. This afforded a precipitate, which was filtered, washed with water, and dried to give **20** in 33% yield (380 mg, 0.369 mmol) after purification by chromatography using gradient of CH_2Cl_2/MeOH from 98/2 to 85/15.

- ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.22–1.45 (m, 10H), 1.34 (s, 6H), 1.86 (m, 2H), 1.99 (s, 3H), 2.32 (t, J = 6.4 Hz, 4H), 2.43 (s, 3H), 2.45 (s, 3H), 2.86 (s, 2H), 3.12 (t, J = 6.9 Hz, 4H), 3.38 (m, 6H), 3.88 (s, 3H), 3.94 (m, 5H), 4.20 (t, 1H), 7.22–7.45 (m, 6H), 7.50 (dd, J_1 = 2.2 Hz, J_2 = 9.3 Hz 1H), 7.62 (m, 4H), 7.73 (d, J = 7.4 Hz, 2H), 8.24 (d, J = 9.3 Hz, 1H).
- ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 12.5, 17.2, 18.4, 19.6, 27.0, 27.4, 28.7, 30.2, 30.7, 36.8, 37.2, 40.1, 41.3, 43.9, 56.2, 56.7, 67.8, 87.6, 104.1, 111.3, 111.8, 115.4, 118.6, 120.8, 121.5, 125.0, 126.3, 126.4, 127.0, 128.1, 128.7, 129.2, 133.4, 135.9, 139.3, 141.4, 141.7, 142.4, 144.9, 145.1, 157.7, 158.1, 159.8, 173.7, 174.7.
- MS (ES) m/z: 1130.5 (MH^+) (100%).

5.1.18. 2-Amino-*N*-(3-{3-[6-(6-chloro-2-methoxyacridin-9-ylamino)hexylamino]-3-oxopropylamino}-3-oxopropyl)-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino]-pentanamide (21). DBU (27 µL, 0.18 mmol) was added to a solution of **20** (200 mg, 0.18 mmol) in THF (10 mL) and the solution was stirred at room temperature for 3 days. The reaction mixture was poured into diethyl ether (100 mL) whilst stirring. The precipitate was filtered, washed with diethyl ether, and dried. It was dissolved in CH_2Cl_2 (100 mL) and the solution was washed with saturated aqueous NaCl (50 mL). After preparative TLC in CH_2Cl_2/MeOH (90/10), acridine **21** was obtained in 32% yield (53 mg, 0.058 mmol).

- ^{1}H NMR (CD_3OD, 400 MHz) δ ppm: 1.30–1.64 (m, 8H), 1.40 (s, 6H), 1.77 (m, 4H), 2.02 (s, 3H), 2.34–2.38 (m, 4H), 2.47 (s, 3H), 2.54 (s, 3H), 2.90 (s, 2H), 3.08–3.15 (m, 4H), 3.38–3.46 (m, 6H), 3.77 (t, J = 7 Hz, 2H), 3.91 (s, 3H), 7.20 (dd, J_1 = 2.0 Hz, J_2 = 9.6 Hz, 1H), 7.36 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.44 (d, J = 2.8 Hz, 1H), 7.72 (d, J = 9.6 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 8.15 (d, J = 9.2 Hz, 1H).
- ^{13}C NMR (CD_3OD, 63 MHz) δ ppm: 12.3, 19.3, 20.0, 27.2, 28.7, 30.3, 31.7, 35.1, 35.6, 38.7, 42.4, 48.2, 49.3, 53.9, 54.8, 86.0, 100.0, 116.9, 122.7, 124.4, 125.7, 128.8, 131.8, 132.3, 132.9, 137.9, 138.3, 155.2, 156.0, 157.8, 158.7, 172.0, 175.2.
- MS (ES) m/z: 454.7 ($M+2H^+$)/2 (100%), 908.2 (MH^+) (94%).

5.1.19. 9-(1-Amino-6-ammonio-1-iminio-7,11,15-trioxo-2,8,12,16-tetraazadocosan-22-ylamino)-6-chloro-2-methoxyacridinium trichloride (22). Et_3SiH (15 µL, 0.08 mmol) was added in the dark to a solution of acridine **21** (37 mg, 0.04 mmol) in TFA/H_2O (90/10) (4 mL). The resulting solution was stirred at room temperature overnight. Deprotection was monitored by reverse phase TLC in AcOH/MeOH/H_2O (2/2/4). The solvents were removed under reduced pressure and the crude residue was washed with methanol, evaporated in vacuo, and dissolved in water (1 mL). This solution was passed through a column of ion exchange resin Dowex Cl^-. The aqueous layer was lyophilized and the product was precipitated in H_2O/acetone to afford acridine **22** as a yellow powder in 70% yield (18 mg, 0.028 mmol).

- ^{1}H NMR (CD_3OD, 250 MHz): 1.50–1.67 (m, 4H), 2.40 (m, 4H), 3.17 (m, 4H), 3.40 (m, 4H), 3.97 (t, 1H), 4.10 (s, 3H), 4.12 (t, J = 2.2 Hz, 2H), 7.53 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.64 (dd, J_1 = 2.8 Hz, J_2 = 7.2 Hz, 1H), 7.80 (d, J = 7.2 Hz, 1H), 7.89 (dd, J_1 = 2.8 Hz, J_2 = 10.8 Hz, 2H), 8.50 (d, J = 9.2 Hz, 1H).
- ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 26.6, 26.7, 27.4, 30.1, 30.6, 34.3, 39.8, 39.9, 40.0, 40.1, 41.7, 56.9, 57.2, 58.4, 110.7, 118.5, 121.4, 121.5, 125.0, 128.6, 128.7, 128.8, 129.3, 129.5, 141.7, 141.8, 158.1, 169.0.
- UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 278 (52,810), 340 (4800), 422 (9920), 440 (9260).
- MS (ES) m/z: 328.8 ($M+2H^+$)/2 (100%), 656.4 (MH^+) (39%).
- Anal. Calcd for $C_{32}H_{49}Cl_4N_9O_4$, NaCl, $3H_2O$: C, 43.77; H, 6.31; N, 14.36. Found C, 43.80; H, 6.41; N, 14.78.

5.1.20. 9-(6-Ammoniohexylamino)-6-chloro-2-methoxyacridinium dichloride (2). Compound **1** (100 mg) was dissolved in TFA (2 mL). The mixture was stirred overnight at room temperature, then TFA was evaporated. The solid was dissolved in distilled water and passed over an ion exchange resin Dowex Cl^-. The chloride salt **2** was obtained quantitatively, after precipitation in a water-acetone mixture and lyophilization.

- UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 276 (30,700), 340 (2375), 422 (5920), 442 (5510).
- MS (ES) m/z: 358.2 (MH^+) (100%).

– Anal. Calcd for $C_{20}H_{26}Cl_3N_3O$, 1.1NaCl, 1.1H_2O: C, 46.65; H, 5.52; N, 8.16. Found C, 46.59; H, 5.49; N, 7.51.

5.1.21. 9-[6-(4-Ammoniobutanamido)hexylamino]-6-chloro-2-methoxyacridinium dichloride (6). Compound **5** was dissolved in distilled water and passed over an ion exchange resin Dowex Cl^-. The chloride salt **6** was obtained quantitatively, after precipitation in a water-acetone mixture and lyophilization. Same treatment applied to compounds **8** and **14** afforded the chloride salts 9-{6-[4-(2-ammonioacetamido)butanamido]hexylamino}-6-chloro-2- methoxyacridinium dichloride (**9**) and 9-[6-(3-ammoniopropanamido)hexylamino]-6-chloro-2-methoxyacridinium dichloride (**15**), respectively.

Acridine **6**

– UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 276 (45,980), 340 (3875), 422 (8540), 442 (8080).
– MS (ES) *m*/*z*: 443.2 (MH^+) (100%).
– Anal. Calcd for $C_{24}H_{33}Cl_3N_4O_2$, HCl, H_2O: C, 50.54; H, 6.36; N, 9.82. Found C, 50.88; H, 6.29; N, 9.89.

Acridine **9**

– UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 278 (36,750), 340 (3010), 420 (7070), 440 (6755).
– MS (ES) *m*/*z*: 500.2 (MH^+) (100%).
– Anal. Calcd for $C_{26}H_{36}Cl_3N_5O_3$, 0.7NaCl, 3H_2O: C, 46.75; H, 6.34; N, 10.49. Found C, 46.74; H, 6.31; N, 11.27.

Acridine **15**

– UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 278 (35,265), 340 (2640), 422 (6780), 442 (6535).
– MS (ES) *m*/*z*: 429.2 (MH^+) (100%).
– Anal. Calcd for $C_{23}H_{31}Cl_3N_4O_2$, 4.3H_2O: C, 47.68; H, 6.89; N, 9.67. Found C, 47.62; H, 7.16; N, 11.55.

5.1.22. 8-(*tert*-Butoxycarbonylamino)caprylic acid (23). Boc-protection of 8-aminocaprylic acid was performed as described for **3**. Compound **23** was obtained as a yellow oil in 90% yield (2.33 g, 9 mmol) and used without further purification.

– ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.30 (m, 8H), 1.40 (s, 9H), 1.60 (m, 2H), 2.30 (t, J = 6.6 Hz, 2H), 3.00 (q, 2H), 4.55 (s, 1H), 5.75 (s, 1H).
– ^{13}C NMR ($CDCl_3$, 50 MHz) δ ppm: 23.9, 26.4, 28.4, 28.8, 28.9, 29.9, 34.0, 40.3, 84.0, 155.9, 169.4.
– MS (ES) *m*/*z*: 258.3 (MH^+) (100%).

5.1.23. *tert*-Butyl 8-(acridin-9-ylamino)-8-oxooctylcarbamate (24). A mixture of **23** (1.65 g, 6.37 mmol), BOP (3.1 g, 7 mmol), and DIEA (1.33 mL, 7.64 mmol) in DMF (25 mL) was stirred at room temperature for 15 min. Then, 9-aminoacridine (6.37 mmol) was added and the reaction mixture was stirred overnight in the dark. The solvent was evaporated and the residue was dissolved in acetone (6 mL), and added dropwise to a stirred solution of 5% $NaHCO_3$ (60 mL). The mixture was allowed to stand for 24 h at room temperature. This afforded a precipitate, which was filtered and purified by MPLC on silica gel (CH_2Cl_2/MeOH, 98/2). Acridine **24** was obtained as an amorphous powder in 64% yield (1.78 g, 4.09 mmol).

– ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.35 (m, 17H), 1.75 (m, 2H), 2.40 (t, 2H), 3.10 (m, 3H), 4.70 (s, 1H), 6.95 (t, J = 7.4 Hz, 2H), 7.25 (t, J = 7.4 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H).
– ^{13}C NMR (DMSO, 63 MHz) δ ppm: 25.3, 26.3, 28.3, 28.5, 28.6, 28.9, 29.6, 36.0, 77.9, 121.7, 122.6, 123.4, 127.9, 130.3, 147.9, 148.9, 150.8, 156.0.
– MS (ES) *m*/*z*: 458.3 (MNa^+) (100%).

5.1.24. 8-(Acridin-9-ylamino)-8-oxooctan-1-ammonium trifluoroacetate (25). Treatment of **24** with TFA (same procedure as for **4**) afforded acridine **25** in quantitative yield.

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.40 (m, 6H), 1.70 (m, 2H), 1.90 (m, 2H), 2.80 (t, 2H), 2.90 (m, 2H), 7.20 (t, 1H), 7.50 (t, 1H), 7.70 (dd, 2H), 8.10 (dd, 2H), 8.30 (d, 1H).
– ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 26.3, 27.3, 28.5, 29.9, 30.1, 37.4, 40.7, 112.3, 119.5, 120.7, 122.7, 124.9, 125.1, 127.2, 128.5, 136.6, 138.3, 140.2, 141.5, 153.4, 175.5.
– MS (ES) *m*/*z*: 336.3 (MH^+) (100%).

5.1.25. *tert*-Butyl 2-[8-(acridin-9-ylamino)-8-oxooctylamino]-2-oxoethylcarbamate (26). Coupling of **25** (324 mg, 0.967 mmol) with Boc-Gly-OH was performed as described for synthesis of **7** and afforded acridine **26** in 38% yield (181 mg, 0.367 mmol).

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.35 (m, 17H), 1.75 (qn, J = 8 Hz, 2H), 2.60 (t, J = 8 Hz, 2H), 3.10 (t, 2H), 3.55 (d, 2H), 7.50 (t, J = 7.4 Hz, 2H), 7.75 (t, J = 7.4 Hz, 2H), 8.00 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.6 Hz, 2H).
– ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 25.4, 26.0, 28.2, 28.3, 28.8, 29.2, 36.4, 39.0, 44.4, 80.3, 122.6, 123.4, 125.9, 126.2, 129.5, 130.2, 149.1, 155.0, 169.7, 173.1.
– MS (ES) *m*/*z*: 515.2 (MNa^+) (100%), 493.2 (MH^+) (32%).

5.1.26. 2-[8-(Acridin-9-ylamino)-8-oxooctylamino]-2-oxoethanammonium trifluoroacetate (27). Acridine **27** was obtained from **26** quantitatively according to the procedure described for the preparation of **5**.

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.50 (m, 8H), 1.88 (m, 2H), 2.88 (t, J = 7 Hz, 2H), 3.25 (t, J = 6.7 Hz, 2H), 3.70 (s, 2H), 7.86 (t, J = 7.4 Hz, 2H), 8.25 (m, 4H), 8.41 (d, J = 8.5 Hz, 2H).
– ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 26.3, 27.7, 30.0, 30.1, 30.3, 37.4, 40.5, 41.4, 112.3, 119.5, 120.7, 122.6, 122.7, 125.1, 127.1, 128.7, 136.6, 138.3, 140.2, 141.6, 151.5, 152.9, 175.5.
– MS (ES) *m*/*z*: 393.2 (MH^+) (100%), 415.2 (MNa^+) (72%).

5.1.27. (9*H*-Fluoren-9-yl)methyl 1-{2-[8-(acridin-9-ylamino)-8-oxooctylamino]-2-oxoethylamino}-1-oxo-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino]pentan-2-ylcarbamate (28). A mixture of Fmoc-Arg(Pbf)-OH (238 mg, 0.367 mmol), BOP (195 mg, 0.44 mmol), and DIEA (76 μL, 0.44 mmol) in DMF (15 mL) was stirred at room temperature for 45 min, then cooled to 0 °C. Acridine **27** (144 mg, 0.367 mmol) and DIEA (320 μL, 1.835 mmol) were dissolved in DMF (5 mL) and added dropwise at 0 °C to the activated ester. The reaction mixture was stirred for 20 min at 0 °C, then overnight at room temperature. After usual work-up, the precipitate was purified by MPLC using gradient of CH_2Cl_2/MeOH from 99/1 to 90/10. Acridine **28** was obtained in 45% yield (170 mg, 0.166 mmol).

– ^{1}H NMR (CD_3OD, 400 MHz) δ ppm: 1.40–1.92 (m, 16H), 2.13 (m, 4H), 2.17 (s, 3H), 2.40 (s, 3H), 2.45 (s, 3H), 2.58 (t, 2H), 2.80 (s, 2H), 3.02 (q, *J*= 5.7 Hz, 4H), 3.22 (q, *J* = 14 Hz, 2H), 3.77 (t, 1H), 4.07 (t, 1H), 4.27 (t, 2H), 7.14 (t, *J* = 7.4 Hz, 2H), 7.24 (t, *J* = 7.1 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 4H), 7.65 (d, *J* = 7.2 Hz, 2H), 7.71 (t, *J* = 7.3 Hz, 2H), 7.98 (d, *J* = 8.7 Hz, 2H), 8.04 (d, *J* = 8.8 Hz, 2H).
– ^{13}C (NMR CD_3OD, 100 MHz) δ ppm: 12.5, 18.4, 19.6, 25.5, 26.3, 28.0, 28.6, 28.7, 28.9, 29.3, 35.8, 39.1, 42.2, 42.5, 46.9, 48.2, 53.4, 66.6, 86.2, 117.0, 122.9, 124.6, 132.1, 132.8, 138.0, 140.4, 141.2, 143.7, 148.8, 156.7, 157.4, 158.4, 169.9, 173.9, 174.7.
– MS (ES) *m*/*z*: 1023.4 (MH^+) (100%).

5.1.28. 1-{2-[8-(Acridin-9-ylamino)-8-oxooctylamino]-2-oxoethylamino}-1-oxo-5-[3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidine]pentan-2-ammonium chloride (29). DBU (100 μL, 0.66 mmol) was added to a solution of acridine **28** (170 mg, 0.166 mmol) in THF (5 mL) and the mixture was stirred at room temperature for 2 h. It was then poured into diethyl ether (100 mL) whilst stirring. The precipitate was filtered, washed with diethyl ether, and dried. It was then dissolved in CH_2Cl_2 (50 mL) and the solution was washed with water (3 × 50 mL), then with saturated aqueous NaCl solution (50 mL), and dried over Na_2SO_4. After evaporation in vacuo, acridine **29** was obtained in quantitative yield.

– ^{1}H NMR (CD_3OD, 250 MHz) δ ppm: 1.20–1.80 (m, 20H), 1.93 (s, 3H), 2.36 (s, 3H), 2.43 (s, 3H), 2.57 (t, *J* = 7.8 Hz, 2H), 2.85 (s, 2H), 3.02 (m, 4H), 3.69 (t, *J* = 4.3 Hz, 1H), 3.85 (q, *J* = 18.4 Hz, 2H), 7.45 (t, *J* = 8 Hz, 2H), 7.70 (t, *J* = 7.4 Hz, 2H), 8.00 (d, *J* = 8.6 Hz, 2H), 8.07 (d, *J* = 8.6 Hz, 2H).
– ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 12.5, 18.4, 19.6, 26.5, 26.8, 27.7, 28.7, 30.0, 30.3, 33.1, 37.1, 40.4, 41.6, 43.3, 43.9, 55.6, 87.6, 118.4, 124.3, 125.1, 125.4, 125.9, 127.3, 129.4, 132.1, 133.4, 134.3, 139.3, 141.7, 150.1, 158.1, 159.8, 171.3, 176.1, 177.8.
– MS (ES) *m*/*z*: 801.5 (MH^+) (100%).

5.1.29. 1-{2-[8-(Acridin-9-ylamino)-8-oxooctylamino]-2-oxoethylamino}-5-[amino(iminio)methylamino]-1-oxopentan-2-ammonium dichloride (30). Acridine **29** (20 mg, 0.025 mmol) was dissolved in TFA/H_2O (95/5) (2 mL) and triethylsilane was added (8 μL, 0.05 mmol). The reaction mixture was stirred overnight at room temperature and the solvent was evaporated. The crude residue was dissolved in water (1 mL) and this solution was passed through a column of ion exchange resin Dowex Cl^-. The aqueous layer was lyophilized and afforded acridine **30** as a yellow powder in quantitative yield (13.8 mg, 0.025 mmol).

– ^{1}H NMR (CD_3OD, 400 MHz) δ ppm: 1.58 (m, 4H), 1.75 (m, 4H), 1.91 (q, *J* = 8 Hz, 4H), 1.99 (q, *J* = 5.6 Hz, 2H), 2.93 (m, 2H), 3.29 (t, *J* = 14 Hz, 4H), 3.85 (s, 2H), 4.05 (t, *J* = 6 Hz, 1H), 7.89 (t, *J* = 7.2 Hz, 2H), 8.25 (t, *J* = 7.2 Hz, 2H), 8.35 (d, *J* = 7.2 Hz, 2H), 8.45 (d, *J* = 7.2 Hz, 2H).
– ^{13}C NMR (CD_3OD, 50 MHz) δ ppm: 25.1, 26.3, 27.7, 29.4, 30.0, 30.2, 37.6, 40.4, 41.8, 43.3, 54.0, 120.9, 123.1, 127.4, 128.8, 138.6, 141.9, 158.5, 170.3, 175.5.
– UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 251 (33,900), 358 (4200).
– MS (ES) *m*/*z*: 549.4 (MH^+) (100%), 275.4 ($M+2H^+$)/2 (56%).
– Anal. Calcd for $C_{29}H_{42}Cl_2N_8O_3$, 1.5 NaCl, $3H_2O$: C, 45.63; H, 6.34; N, 14.68. Found C, 45.52; H, 6.51; N, 13.87.

5.1.30. Compound 31
– UV–vis (H_2O): λ_{max} nm (ε mol^{-1} L cm^{-1}) = 251 (88,300), 358 (7600).
– MS (ES) *m*/*z*: 535.3 (MH^+) (100%), 268.2 ($M+2H^+$)/2 (90%). MS (HRMS) *m*/*z* calcd for $C_{28}H_{39}N_8O_3$ (MH^+): 535.3145; found: 535.3151.
– Anal. Calcd for $C_{28}H_{40}Cl_2N_8O_3$, NaCl, $2H_2O$: C, 47.90; H, 6.32; N, 15.96. Found C, 47.78; H, 6.29; N, 15.46.

5.2. Biology

5.2.1. *P. falciparum* strains. Both CQ-sensitive (3D7) and CQ-resistant (W2, FCR3, and Bre1) *P. falciparum* strains maintained continuously in culture were used. Synchronous parasites were diluted with uninfected erythrocytes (A-positive human blood) and completed RPMI 1640 medium (Invitrogen, Paisley, United Kingdom), supplemented with 10% human serum Abcys S.A. (Paris, France) and buffered with 25 mM HEPES and 25 mM $NaHCO_3$ to achieve 0.2 parasitemia and 1.5 hematocrit.

5.2.2. Measurement of in vitro antimalarial activity. Solutions of drugs were prepared in RPMI 1640 medium and distributed in triplicate into Falcon 96-well flat-bottomed plates (Becton Dickinson, Franklin Lakes, NJ) to achieve concentrations ranging from 0.06 μM to 200 μM.

For in vitro isotopic microtests, 200 μL/well of the suspension of parasitized erythrocytes was distributed in 96-well plates predosed with antimalarial agents. Parasite growth was assessed by adding 1 μCi of [^{3}H]hypoxanthine with a specific activity 14.1 Ci/mmol (NEN Products, Dreiech, Germany) to each well. Plates were incubated for 42 h at 37 °C in an atmosphere of 10%

O_2, 5% CO_2, 85% N_2, and an humidity of 95%. Immediately after incubation, the plates were frozen and then thawed to lyse erythrocytes. The contents of each well were collected on standard filter microplates (Unifilter™ GF/B, Perkin Elmer, Meriden, USA) and washed using a cell harvester (FilterMate™ Cell Harvester, Packard). Filter microplates were dried and 25 μL of scintillation cocktail (Microscint™ O, Perkin Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured using a scintillation counter (Top Count™, Perkin Elmer).

The 50% inhibitory concentration (IC_{50}), that is, the drug concentration corresponding to 50% of the uptake of [^{3}H]hypoxanthine by the parasites in drug-free control wells, was determined by non-linear regression analysis of log-dose/response curves (Riasmart™, Packard, Meriden, USA). Data were analyzed after logarithmic transformation and expressed as the geometric mean IC_{50} and 95% confidence intervals (95% CI) were calculated (Stata9™, StataCorp LP, Texas, USA).

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References and notes

1. Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5274–5293.
2. Winstanley, P. A.; Breckenridge, A. M. *Ann. Trop. Med. Parasitol.* **1987**, *81*, 619–627.
3. Girault, S.; Delarue, S.; Grellier, P.; Berecibar, A.; Maes, L.; Quirijnen, L.; Lemiere, P.; Debreu Fontaine, M. A.; Sergheraert, C. *J. Pharm. Pharmacol.* **2001**, *53*, 935–938.
4. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemiere, P.; Debreu, M. A.; Davioud Charvet, E.; Sergheraert, C. *J. Med. Chem.* **2000**, *43*, 2646–2654.
5. Santelli Rouvier, C.; Pradines, B.; Berthelot, M.; Parzy, D.; Barbe, J. *Eur. J. Med. Chem.* **2004**, *39*, 735–744.
6. Anderson, M. O.; Sherrill, J.; Madrid, P. B.; Liou, A. P.; Weisman, J. L.; DeRisi, J. L.; Guy, R. K. *Bioorg. Med. Chem.* **2006**, *14*, 334–343.
7. Chavalitshewinkoon, P.; Wilairat, P.; Gamage, S.; Denny, W.; Figgitt, D.; Ralph, R. *Antimicrob. Agents Chemother.* **1993**, *37*, 403–406.
8. Elueze, E. I.; Croft, S. L.; Warhurst, D. C. *J. Antimicrob. Chemother.* **1996**, *37*, 511–518.
9. Figgitt, D.; Denny, W.; Chavalitshewinkoon, P.; Wilairat, P.; Ralph, R. *Antimicrob. Agents Chemother.* **1992**, *36*, 1644–1647.
10. Shibnev, V. A.; Finogenova, M. P.; Grinberg, L. N.; Allakhverdiev, A. M. *Bioorg. Khim.* **1988**, *14*, 1565–1569.
11. Wainwright, M. *J. Antimicrob. Chemother.* **2001**, *47*, 1–13.
12. Hahn, F. E.; Fean, C. L. *Antimicrob. Agents Chemother.* **1969**, *9*, 63–66.
13. Allison, J. L.; O'Brien, R. L.; Hahn, F. E. *Antimicrob. Agents Chemother.* **1965**, *5*, 310–314.
14. Hahn, F. E. *Antibiotics* **1974**, *3*, 58–78.
15. Gamage, S. A.; Tepsiri, N.; Wilairat, P.; Wojcik, S. J.; Figgitt, D. P.; Ralph, R. K.; Denny, W. A. *J. Med. Chem.* **1994**, *37*, 1486–1494.
16. Auparakkitanon, S.; Wilairat, P. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 406–409.
17. Zwelling, L. A.; Mitchell, M. J.; Satitpunwaycha, P.; Mayes, J.; Altschuler, E.; Hinds, M.; Baguley, B. C. *Cancer Res.* **1992**, *52*, 209–217.
18. Choi, C. Y. H.; Schneider, E. L.; Kim, J. M.; Gluzman, I. Y.; Goldberg, D. E.; Ellman, J. A.; Marletta, M. A. *Chem. Biol.* **2002**, *9*, 881–889.
19. Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. *J. Med. Chem.* **2002**, *45*, 4975–4983.
20. Chou, A. C.; Fitch, C. D. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 422–427.
21. Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727–736.
22. Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. *Nature* **2000**, *404*, 307–310.
23. Far, S.; Kossanyi, A.; Verchère-Béaur, C.; Gresh, N.; Taillandier, E.; Perrée-Fauvet, M. *Eur. J. Org. Chem.* **2004**, 1781–1797.
24. Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E.-S. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829–7832.
25. Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748–5749.
26. Auparakkitanon, S.; Noonpakdee, W.; Ralph, R. K.; Denny, W. A.; Wilairat, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3708–3712.
27. Chavalitshewinkoon Petmitr, P.; Pongvilairat, G.; Auparakkitanon, S.; Wilairat, P. *Parasitol. Int.* **2000**, *48*, 275–280.
28. Chavalitshewinkoon Petmitr, P.; Pongvilairat, G.; Ralph, R. K.; Denny, W. A.; Wilairat, P. *Trop. Med. Int. Health* **2001**, *6*, 42–45.
29. Denny, W. A. *Curr. Med. Chem.* **2002**, *9*, 1655–1665.
30. Fukui, K.; Tanaka, K. *Nucleic Acids Res.* **1996**, *24*, 3962–3967.
31. Guetzoyan, L.; Steenkeste, K.; Ramiandrasoa, F.; Perrée-Fauvet, M.; Fontaine-Aupart, M. -P.; Tfibel, F., Results to be published.
32. Seeman, N. C.; Rosenberg, J. M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 804–808.
33. Moreau, S.; Perly, B.; Chachaty, C.; Deleuze, C. *Biochim. Biophys. Acta* **1985**, *840*, 107–116.
34. Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283–291.
35. Ishikawa, Y.; Yamashita, A.; Uno, T. *Chem. Pharm. Bull.* **2001**, *49*, 287–293.
36. Kossanyi, A.; Mestre, B.; Perrée-Fauvet, M. *Synthetic Commun.* **1999**, *29*, 4341–4346.