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Antimalarial in-vivo activity of bis(9-amino-6-chloro-2-methoxyacridines)

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Abstract

In the fight against malaria, chemotherapy using bisacridines may represent an alternative method to overcoming chloroquine-resistance. Eight bis(9-amino-6-chloro-2-methoxyacridines), in which acridine moieties were linked by polyamines substituted with a side chain, were tested for their in-vivo activity upon mice infected by *Plasmodium berghei*. Three of the compounds revealed antimalarial activity but no relationship could be deduced from a comparison of in-vitro and in-vivo activities. *N*-alkylation of the central amino group generated toxicity and, therefore, only compounds *N*-acylated in this position can be selected as leads.

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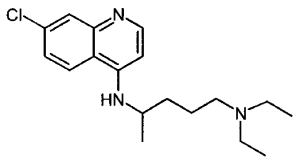
Introduction

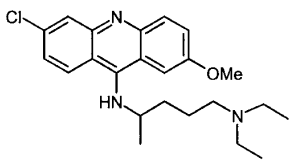
The mainstream drug in the fight against malaria for over 50 years, chloroquine (Figure 1), which is believed to exert its activity by inhibiting haemozoin formation in the digestive vacuole of the malaria parasite (Dorn et al 1995, 1998), is having its efficacy eroded by the emergence of resistant parasites (White 1992; Van Est et al 1993). While this resistance may involve several mechanisms, its reversal by molecules such as verapamil, desipramine and chlorpromazine suggests involvement of an enhanced chloroquine efflux by a multidrug-resistant mechanism (Krogstad et al 1987; Warhurst 1997; Reed et al 2000). One possibility to overcome this mechanism is to design quinoline-based drugs which are not recognized by the proteins involved in drug efflux. In this regard, bulky bisquinolines were synthesized and appeared to be extruded with difficulty by a proteinaceous transporter (Vennerstrom et al 1992; Ismail et al 1996, 1998). They were discovered to inhibit the growth of chloroquine-sensitive and chloroquine-resistant parasites with similar efficacy (Vennerstrom et al 1992; Raynes et al 1995; Cowman et al 1997) but further development was suspended for reasons of toxicity (Ridley et al 1997).

Quinacrine, the 9-amino-6-chloro-2-methoxyacridine analogue of chloroquine (Figure 1), which was used clinically before chloroquine, shares the same features as a weak diprotic base; it accumulates in the acidic food vacuole (pH = 5) of *Plasmodium* and prevents haematin polymerisation (Dorn et al 1998). Until now, bisacridines have been poorly studied for their antiparasitic activity because of their cytotoxic effects, yet they, along with bisquinolines, may represent an alternative method for avoiding the efflux mechanism.

From the hypothesis that bulky structures are extruded with difficulty by chloroquine-resistant strains of *Plasmodium falciparum*, a series of bisacridine



chloroquine

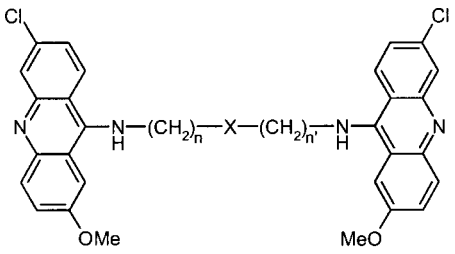


quinacrine

Figure 1 Chloroquine and quinacrine.

derivatives (aliphatic di-, tri-, and tetramine) was prepared (Figure 2; Girault et al 2000). A side chain comprising a variety of amino-acid residues was also attached to the polyamine linker, both to improve the weak solubility of this type of compound and to reduce its possible interaction with human DNA.

The majority of these compounds displayed an IC50 (concentration causing 50 % inhibition of the parasite growth) value between 17 and 500 nM upon FcB1R *P. falciparum* strain (IC50 chloroquine = 126 nM) and cytotoxic effects upon MRC-5 cells and mouse peritoneal macrophages. Only compound **1** (Figure 2) displayed a very high activity (IC50 = 8–18 nM against *P. falciparum* strains showing different degrees of chloroquine-resistance) while being totally devoid of cytotoxicity (Girault et al 2000). Six compounds of this series were selected for their cytotoxicity/activity ratio and tested for their in-vivo activity upon mice infected by *Plasmodium berghei*, as well as two additional compounds **7** and **8** (Figure 2; **7**: n = n′ = 2, X = N-CO-CH_2-NHBoc; **8**: n = n′ = 2, X = N-CH_2-CH_2-NHBoc). Compounds **7** and **8** were synthesized with the aim of explaining the influence of the bond between the side chain and the polyamine linker upon antimalarial activity and cytotoxicity.



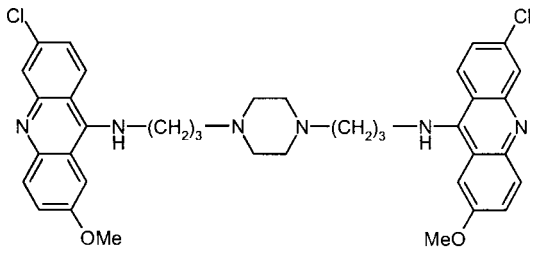


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Figure 2 Bisacridines.

Materials and Methods

Chemical synthesis

Syntheses of compounds **1–6** have been previously published (Girault et al 2000).

NMR experiments

^{1}H and ^{13}C NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) were expressed in ppm relative to TMS (tetramethylsilane) used as an internal standard.

Mass spectrometry

Mass spectra were recorded on a time-of-flight (TOF) plasma desorption spectrometer using a Californium source.

High-pressure liquid chromatography

The purity of final compounds was verified by two types of high-pressure liquid chromatography (HPLC): C18 nucleosil (C18) and C18 nucleosil cyano (CN) columns. Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in ethanol and injected through a 50-μL loop. The following eluent systems were used: A (H_2O–TFA, 100:0.05) (trifluoroacetic acid) and B (CH_3CN–H_2O–TFA, 80:20:0.05). HPLC retention times (HPLC t_R) were obtained, at flow rates of 1 mL min^{-1}, using the following conditions: a gradient run from 100 % eluent A for 5 min, then to 100 % eluent B over the next 25 min.

Synthesis of compounds 7 and 8

N,N-*Bis{3-[*N-*(6-chloro-2-methoxy)acridin-9-yl]aminopropyl}-2-[*N-*(*tert-*butoxycarbonyl)amino]ethanamide (7)*

Compound **7** was synthesized from Boc-Gly-OH according to the method described for compound **10**

Table 1 In-vivo antimalarial activity of bisacridines 1–8.

Molecule	n	n′	X[a]	Excess mean survival time (%)
1	3	3	Piperazine	–[b]
2	3	3	N-CO-CH(NHBoc)-CH_3 (D enantiomer)	0
3	3	3	N-CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$	0
4	3	3	N-CO-CH(NHBoc)-CH_2OAc	0
5	3	3	N-CO-CH(NHBoc)-CH_2-COOH	11
6	3	3	N-CO-$(CH_2)_2$-COOH	8
7	2	2	N-CO-CH_2-NHBoc	22
8	2	2	N-CH_2-CH_2-NHBoc	0

[a]Ac, acetyl; Boc, *tert*-butoxycarbonyl. [b]Lack of solubility.

(Girault et al 2000) and isolated as an orange oil (60 % yield); R_f 0.3 (CH_2Cl_2–MeOH, 9:1); HPLC (CN) purity determined by HPLC (P_{HPLC}) 96.5 %, t_R 23.6 min; HPLC (C18) P_{HPLC} 95.7 %, t_R 27.5 min; ^{1}H NMR ($CDCl_3$–CD_3OD, 2:1) δ 8.04–8.00 (m, 1 H, Ar-H), 7.74–7.70 (m, 1 H, Ar-H), 7.55–7.32 (m, 5 H, Ar-H), 7.30–7.24 (m, 2 H, Ar-H), 7.15–7.14 (m, 1 H, Ar-H), 7.09–7.05 (m, 1 H, Ar-H), 6.90–6.86 (m, 1 H, Ar-H), 4.31–4.17 (m, 2 H, CH_2), 4.13–4.00 (m, 4 H, 2 CH_2), 3.99 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 2.73–2.64 (m, 4 H, 2 CH_2), 1.49 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$–CD_3OD, 2:1) δ 127.55, 127.10, 125.39, 125.02, 124.20, 122.86, 121.53, 118.54, 101.10, 56.11, 55.89, 53.99, 52.23, 50.60, 31.47, 29.79, 28.42, 27.80, 27.74; TOFMS m/z 743 (M^+).

*Bis{2-[*N*-(6-chloro-2-methoxyacridin-9-yl)amino]ethyl}-{2-[*N*-(*tert*-butoxycarbonyl)amino]ethyl}amine (**8**)*

To a solution of 6,9-dichloro-2-methoxyacridine (2.08 g, 7.5 mmol, 3 equiv.), and K_2CO_3 (3.45 g, 25 mmol, 10 equiv.), in 15 mL of DMF (dimethylformamide) was added tris(2-aminoethyl)amine (374 μL, 2.5 mmol, 1 equiv.). Following reflux of the mixture for 4 h, the solvent was evaporated and the solid residue treated with a CH_2Cl_2–H_2O mixture. To a solution of the crude product (2.5 mmol, 1 equiv.) in 10 mL of dioxane was introduced a solution of NaOH (230 mg, 5.75 mmol, 2.3 equiv.) in 6 mL of water, then, di-*tert*-butyldicarbonate (600 mg, 2.75 mmol, 1.1 equiv.). After stirring the mixture at room temperature for 12 h, the dioxane was evaporated and the aqueous residue treated with a CH_2Cl_2–H_2O mixture. The organic layer was dried over $MgSO_4$, the solvent evaporated and the oily residue purified by thick-layer chromatography (TLC) (CH_2Cl_2–MeOH, 90:10), to yield compound **8** as an orange solid (30 % yield); R_f 0.7 (CH_2Cl_2–MeOH, 9:1); mp 65–67°C; HPLC (CN) P_{HPLC} 98.9 %, t_R 24.0 min; HPLC (C18) P_{HPLC} 98.9 %, t_R 27.9 min; ^{1}H NMR ($CDCl_3$–CD_3OD, 2:1) δ 7.97 (d, J = 9.3 Hz, 2 H Ar-H), 7.68–7.63 (m, 4 H, Ar-H), 7.29–7.25 (m, 2 H, Ar-H), 7.18–7.16 (m, 2 H, Ar-H), 7.08–7.03 (m, 2 H, Ar-H), 3.86–3.79 (m, 10 H, 2 CH_2 and 2 OCH_3), 3.38–3.27 (m, 2 H, CH_2), 2.96 (t, J = 5.5 Hz, 4 H, 2 CH_2), 2.86–2.77 (m, 2 H, CH_2), 1.41 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$–CD_3OD, 2:1) δ 127.91, 125.35, 125.13, 124.77, 123.90, 100.28, 55.60, 54.82, 47.65, 38.98, 28.46; TOFMS m/z 729 (M^+).

Biological evaluation

In-vivo drug assays upon P. berghei

Antimalarial in-vivo activities were determined in mice infected with *P. berghei* (ANKA 65 strain). Four-week-old female Swiss mice (CD-1, 20–25g) were intraperitoneally infected with about 10^7 parasitized erythrocytes, collected from the blood of an acutely infected donor mouse. At the same time, the mice (3 per group) were orally treated with the test compound at 40 mg kg^{-1} (drug formulation in 100 % DMSO; dimethylsulfoxide). The treatment was continued for the next 4 days by the intraperitoneal route. Untreated control mice generally died 7–10 days after infection. Drug activity was evaluated as the prolongation of the mean survival time observed with untreated controls. Three infected DMSO-dosed mice were used as controls.

Results and Discussion

Molecules **1–8** were tested, in-vivo, at 40 mg kg^{-1}, upon mice infected with *P. berghei* (Table 1).

Chloroquine, used as a control, led to an increase in mean survival time of more than 120% at 10 mg kg^{-1}. Lack of solubility of compound **1** in DMSO prevented its being tested, while the other compounds most active in-vitro (**2–4**) were found to be inactive at 40 mg kg^{-1},with the mice dying 7–10 days following infection, like untreated controls. Only compounds **5–7** increased the mean survival time, while with compound **8**, the alkyl analogue of compound **7**, the mice died after three or four days, suggesting toxicity. This in-vivo toxicity of the sole *N*-alkyl compound in the series can be explained by the presence of a cationic site likely to interact with the phosphate groups of DNA. Unexpectedly, compound **7** was the most active compound in-vivo while its close analogues, compounds **2** and **3** (more potent in-vitro), were totally devoid of activity.

These results confirm that it is always difficult to predict relationships between in-vitro and in-vivo activity and toxicity, even for members of the same family of compounds. However, given the convenience of the chemistry alone, the preparation, in parallel, of new analogues of compounds **6** and **7**, with terminal Boc-amino or carboxylic groups, was justified. Equally, to improve its solubility, derivatization of compound **1**, the most promising compound of the series in the in-vitro studies, is also planned.

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