New Potential Antimalarial Agents: Synthesis and Biological Activities of Original Diaza-analogs of Phenanthrene

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Several diaza-analogs of phenanthrene derived from 3-amino, 5-amino, 6-amino, 8-aminoquinolines, and 5-aminoisoquinoline were prepared to evaluate their antiplasmodial activities. All compounds showed mild to good activity *in vitro*, both on a Nigerian chloroquino-sensitive strain and on the chloroquino-resistant FcB1-Columbia and FcM29 strains. The position of the intracyclic nitrogen atom is shown to be crucial for the activities (best results are obtained with a 1,10-phenanthroline skeleton). In regard to the particular properties of this structure (metalloprotease inhibition activity by chelating divalent metal ions), the potential chelating site of the molecule was blocked. In this case, the biological activity of the compound was greatly enhanced, showing that the mechanism of action of such a compound is probably not correlated to metalloprotease inhibition activity.

Key words 1,10-phenanthroline; antiplasmodial; quinoline; Plasmodium falciparum; *in vitro*

Malaria is endemic throughout much of the tropics, and sub-tropics placing at risk some 40% of the world's population. More than 100 million clinical cases of the disease are thought to occur annually, resulting in at least 1—2 million deaths. In addition, since resistance to currently used antimalarials is spreading rapidly, there is a great need for new effective drugs. As part of our program concerning the synthesis and biological activities of aza-analogs of alkaloids,¹⁾ we were interested in the chemistry of diaza-analogs of phenanthrene (phenanthrene alkaloids are obtained from various families of the Angiospermae.²⁾ An example of particular interest of a non-natural antimalarial agent possessing a phenanthrolic skeleton is halofantrine, which possesses good therapeutic effects but some important side effects.³⁾ In addition, heterocycles with two nitrogen atoms, such as pyronaridine⁴⁾ (a benzo[*b*]-1,5-naphthyridine skeleton), are well-known for their antimalarial activities. In this context, we describe here our first results concerning the preparation and *in vitro* biological activities of original diaza-analogs of phenanthrene (compounds **A** and **B**), which can be further implicated in the preparation of new potent antimalarial agents (Chart 1).

Chemistry Our strategy for the synthesis of the tricyclic derivatives of quinoline or isoquinoline possessing an additional pyridinic ring is focused on the use of heterocyclic enamines as synthetic intermediates,⁵⁾ which can then undergo regioselective heterocyclizations. Treatment of the different aminoquinolines **1—4** and aminoisoquinoline **5** by a 1.5 eq of 2-acetylbutyrolactone in refluxing toluene with a catalytic amount of *p*-toluenesulfonic acid gave the corresponding furanones **6—10** in moderate to good yield (Chart 2).

By treatment with phosphorus oxychloride (1 g of **6—10**, 10 ml of freshly distilled POCl₃), these synthetic intermediates underwent regioselective cyclizations and subsequent ring and side chlorination to give **11—15** (Chart 3).⁶⁾

Structural determinations of these pyrido(iso)quinolines

Chart 2

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12–15 and benzonaphthyridines **11** were made on the basis of mass spectrometry and NMR studies. Regioselectivity of the cyclizations was easily determined by simple examination of ^1H - and ^{13}C -NMR data, except for **12**. In this case, examination of ^1H - ^{13}C long-range correlations was necessary to confirm the position of cyclization on the C-6 position of the quinoline ring rather than on the C-4 position.

Results and Discussion

Three strains of *P. falciparum* were used to evaluate the *in vitro* antiparasmodial activities of compounds **11–15**: the chloroquino-resistant FcB1-Columbia and FcM29-Cameron strains and a Nigerian chloroquino-sensitive strain.⁷⁾ Para-

Chart 3

(i) CH_3I , acetone, reflux 12h
(ii) HCl gas, acetone

Chart 4

sites were cultured according to the method originally described by Trager and Jensen.⁸⁾ For each test, the parasite cultures were incubated with compounds at decreasing concentrations for 24 and 72 h. Results are summarized in Table 1. This first screening showed that all compounds were less active on the chloroquino-sensitive strain than chloroquine itself. Compound **14** displayed interesting antiplasmodial activity on the two chloroquino-resistant strains. Compound **11**, possessing a benzonaphthyridine skeleton, was the least active compound, especially after 24 h of contact between drug and parasite, with all the three strains. The three other compounds **12**, **13**, **15**, possessing as **14** a phenanthroline skeleton, exhibited mild activity. These results clearly indicate the primordial effect of the position of the intracyclic nitrogen atom: when the two pyridinic rings are joined together, only slight activity was observed (compound **11**). When the two pyridinic rings are joined toward a phenyl ring (phenanthrolines), the activities increased, especially in the case of the 1,10-phenanthroline ring system.

As the 1,10-phenanthroline ring system is well-known for its metalloprotease inhibition activities by chelating divalent metal ions,⁹⁾ we could reasonably suppose that this phenomenon was implicated in the mechanism of action. In order to obtain such informations, we decided to block the potential chelating site by *N*-alkylation (Chart 4).

Surprisingly, compound **16** exhibited better activity than **14** on the three strains, both after 24 and 72 h incubation (Table 1). This result clearly demonstrated that the metalloprotease inhibition process was not correlated with the mechanism of action against *P. falciparum*. Furthermore, as **16** was more soluble in the aqueous biological phase, it was interesting to investigate the activities of the simple hydrosoluble phenanthroline hydrochloride salt **17**. In this case, antiparasmodial activity was not enhanced, showing that alkylation of the N-10 atom was essential to enhance the activities of such compounds.

Cytotoxic activities of these compounds (**11–17**) were also evaluated *in vitro* against the HeLa cell line. Results are summarized in Table 2. All compounds exhibited cytotoxic effects which were higher than chloroquine but lower than halofantrine. Moreover, these cytotoxic effects increased with antiparasmodial activities for **11–15**, while remaining close to compound **14** for derivatives **16** and **17**. A cytotoxic/antiparasmodial ratio (CAR.) was calculated at 72 h (Table 2), and showed an interesting value (higher than 100¹⁰⁾ for com-

Table 1. IC_{50} (μM) of Compounds **11–17** on the Three *P. falciparum* Strains Tested

	Nigerian		FcB1		FcM29	
IT ^{a)}	24	72	24	72	24	72
11	72.5 $\pm$ 13.4	22.6 $\pm$ 7.5	60.6 $\pm$ 14.0	18.5 $\pm$ 17.8	68.1 $\pm$ 10.3	25.3 $\pm$ 3.8
12	24.9 $\pm$ 7.2	8.9 $\pm$ 7.2	16.8 $\pm$ 3.7	7.5 $\pm$ 4.5	37.7 $\pm$ 20.9	6.9 $\pm$ 3.0
13	17.4 $\pm$ 7.5	13.4 $\pm$ 5.1	27.4 $\pm$ 7.2	14.0 $\pm$ 4.5	18.5 $\pm$ 13.5	7.5 $\pm$ 11.6
14	2.4 $\pm$ 0.4	1.5 $\pm$ 0.6	2.4 $\pm$ 0.8	1.3 $\pm$ 1.1	2.3 $\pm$ 0.4	1.8 $\pm$ 0.3
15	15.7 $\pm$ 6.1	14.4 $\pm$ 3.8	18.8 $\pm$ 4.1	16.4 $\pm$ 6.8	10.6 $\pm$ 3.1	11.3 $\pm$ 6.5
16	0.19 $\pm$ 0.05	0.16 $\pm$ 0.08	0.17 $\pm$ 0.07	0.02 $\pm$ 0.02	0.21 $\pm$ 0.02	0.10 $\pm$ 0.07
17	2.33 $\pm$ 0.70	1.50 $\pm$ 0.50	2.27 $\pm$ 0.03	1.81 $\pm$ 0.06	22.1 $\pm$ 18.2	16.3 $\pm$ 13.4
Hal ^{b)}	0.003	0.0025	0.0034	0.0027	0.0031	0.0028
CQ ^{b)}	0.076	0.076	0.145	0.145	0.210	0.210

a) Incubation time (h), b) Hal=halofantrine, CQ=chloroquine, values checked monthly.

Table 2. IC₅₀ and CAR Values on FcB1 of Compounds **11**–**17** on HeLa Cell Line

	IC ₅₀ (μM)		CAR ^{a)}
IT ^{b)}	24	72	///
11	47.9±13.9	44.1±17.8	2.38
12	26.7±7.2	33.9±11.3	4.52
13	42.1±23.3	46.2±18.1	3.30
14	17.4±3.8	14.0±6.2	10.77
15	52.4±13.4	48.6±6.8	2.96
16	19.6±8.0	15.1±1.8	755.00
17	14.8±0.5	13.4±1.01	2.96
Hal	4.28±0.75	7.26±1.17	2680.80
CQ	68.61±7.85	75.15±9.13	518.27

a) Cytotoxic/antiplasmodial ratio on FcB1 calculated for 72 h of incubation time.

b) Incubation time (h).

pound **16** (CAR_{FcB1}=755, CAR_{FcM29}=151). These CAR values for **16** were obtained by an increasing antiplasmodial effect associated with stable cytotoxicity when compared to compound **14**.

Conclusion

We have described the synthesis and evaluation of a five models of diaza-analogs of the phenanthrene skeleton. From this evaluation, the 1,10-phenanthroline ring system appeared as a new class of potential antimalarial compounds. From these results, and with respect to the particular properties of this skeleton, our first hypothesis about a possible mechanism of action led us to consider a new derivative (compound **16**) which exhibited higher antiplasmodial activity, with similar cytotoxic activity. Further studies concerning the pharmacomodulation, the mechanism of action, and the *in vivo* evaluation of such compounds are now underway.

Experimental

Melting points were determined on a Büchi capillary melting point apparatus and are not corrected. Elemental analysis was performed by the Micro-analytical Center, ENSCM, Montpellier. Spectral measurements were taken using the following instruments: ¹H-NMR spectra were taken on Brüker AC 100 or WM 360 or EM 400WB; ¹³C-NMR spectra were obtained at 26 °C, with proton noise decoupling at 25 MHz with a Brüker AC 100 instrument. Chemical shifts are expressed relative to residual chloroform. Mass spectra were recorded on an LKB 2091 spectrometer at 15 eV [θ(source)=180 °C]. Dichloromethane was dried over activated alumina and distilled from calcium hydride.

3-[1-(3-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (6) A mixture of 2-acetyl-butylolactone (1 g, 8 mmol) and 3-aminoquinoline (**1**) (1.15 g, 8 mmol), and a catalytic amount of *p*-toluenesulfonic acid in toluene (25 ml), was refluxed under nitrogen with a Dean Stark apparatus for 6 h. After evaporation of the solvent, water was added and the mixture was basified (10% Na₂CO₃), saturated with NaCl, and extracted with dichloromethane. Organic layers were washed with brine (50 ml), dried over sodium sulfate and evaporated *in vacuo*. The remaining oil was treated with ether, cooled, and the precipitate was filtered. Recrystallization from ethanol gave (**6**) in 90% yield; mp 150–152 °C. MS (relative intensity) *m/z*: 254 (M⁺, 96), 195 (50), 128 (53). ¹H-NMR (CDCl₃) δ: 2.07 (s, 3H), 2.91 (2H, t, *J*=7.0 Hz), 4.35 (2H, t, *J*=7.0 Hz), 7.61 (4H, m), 8.06 (1H, d, *J*=7.4 Hz), 8.65 (1H, d, *J*=2.5 Hz), 10.15 (1H, s). ¹³C-NMR (CDCl₃) δ: 17.4 (CH₃), 25.9, 65.2, 91.7, 126.2, 126.9, 127.0, 127.6, 128.1, 128.7, 132.5, 144.8, 147.3, 151.9, 173.48. Anal. Calcd for C₁₅H₁₄N₂O₂: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.69; H, 5.65; N, 11.12.

3-[1-(5-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (7) This compound was obtained in 88% yield from 5-aminoquinoline (**2**) according to the general procedure used for the synthesis of **6**; mp 116–118 °C. MS (relative intensity) *m/z*: 254 (M⁺, 97), 195 (53), 128 (50). ¹H-NMR (CDCl₃) δ: 1.79 (3H, s), 2.81 (2H, t, *J*=8 Hz), 4.27 (2H, t, *J*=8 Hz), 7.38 (4H, m), 7.80 (1H, d, *J*=9 Hz), 8.23 (1H, d, *J*=8 Hz), 8.78 (1H, d, *J*=5

Hz), 10.15 (1H, s). ¹³C-NMR δ: 17.4, 25.1, 65.4, 90.24, 121.30, 122.7, 124.1, 127.1, 128.6, 130.9, 134.9, 148.6, 150.5, 154.0, 174.05. Anal. Calcd for C₁₅H₁₄N₂O₂: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.95; H, 5.51; N, 11.32.

3-[1-(6-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (8)

This compound was obtained in 80% yield from 6-aminoquinoline (**3**) according to the general procedure used for the synthesis of **6**; mp 128–130 °C. MS (relative intensity) *m/z*: 254 (M⁺, 97), 209 (54), 169 (39). ¹H-NMR (CDCl₃) δ: 2.10 (3H, s), 2.90 (2H, t, *J*=8.0 Hz), 4.35 (2H, t, *J*=8.0 Hz), 7.26 (3H, m), 7.92 (2H, d, *J*=11.0 Hz), 8.78 (1H, d, *J*=4.1 Hz), 10.18 (1H, s). ¹³C-NMR δ: 17.3, 25.7, 64.81, 90.62, 118.1, 121.1, 125.9, 128.0, 129.8, 134.6, 136.7, 144.8, 148.8, 151.9, 173.2. Anal. Calcd for C₁₅H₁₄N₂O₂: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.69; H, 5.69; N, 11.00.

3-[1-(8-Quinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (9)

This compound was obtained in 85% yield from 8-aminoquinoline (**4**) according to the general procedure used for the synthesis of **6**; mp 130–132 °C. MS (relative intensity) *m/z*: 254 (M⁺, 53), 209 (84), 169 (96). ¹H-NMR (CDCl₃) δ: 2.24 (3H, s), 2.98 (2H, t, *J*=8.0 Hz), 4.34 (2H, t, *J*=8.0 Hz), 7.33 (4H, m), 8.13 (1H, d, *J*=8.2 Hz), 8.91 (1H, d, *J*=3.7 Hz), 11.39 (1H, s). ¹³C-NMR δ: 15.4, 26.8, 65.2, 92.81, 117.4, 121.6, 121.8, 121.9, 126.3, 128.8, 135.9, 136.8, 149.3, 151.3, 174.5. Anal. Calcd for C₁₅H₁₄N₂O₂: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.89; H, 5.39; N, 10.89.

3-[1-(5-Isoquinolin-3-ylamino)-ethylidene]-4,5-dihydrofuran-2-one (10)

This compound was obtained in 83% yield from 5-aminoisoquinoline (**5**) according to the general procedure used for the synthesis of **6**; mp 126–128 °C. MS (relative intensity) *m/z*: 254 (M⁺, 97), 226 (30), 195 (53). ¹H-NMR (CDCl₃) δ: 1.79 (3H, s), 2.80 (2H, t, *J*=8.0 Hz), 4.25 (2H, t, *J*=8.0 Hz), 7.48 (4H, m), 8.37 (1H, d, *J*=6.0 Hz), 9.09 (1H, s), 10.13 (1H, s). ¹³C-NMR δ: 17.6, 26.3, 65.6, 90.8, 115.4, 125.3, 126.2, 126.8, 129.4, 132.6, 134.5, 143.8, 152.8, 153.9, 174.3. Anal. Calcd for C₁₅H₁₄N₂O₂: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.98; H, 5.49; N, 11.22.

1-Chloro-2-(2-chloroethyl)-3-methylbenzo[*f*][1,7]naphthyridine (11)

Compound (**6**) (1 g, 4 mmol) was heated with phosphorus oxychloride (POCl₃, 25 ml) until the end of the exothermic reaction (80–90 °C). The mixture was then refluxed for 4 h. Excess phosphorus oxychloride was removed, water was added and the mixture was neutralized with a saturated solution of sodium carbonate. After extraction with dichloromethane, the organic layers were washed with brine (50 ml), then dried (Na₂SO₄). Solvent evaporation gave a black oily product which was purified on a neutral alumina and eluted with dichloromethane to afford 0.87 g (75%) of **11**; mp 146–148 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M⁺, 55), 241 (97), 243 (35). ¹H-NMR (CDCl₃) δ: 2.98 (3H, s), 3.56 (2H, t, *J*=7.0 Hz), 3.68 (2H, t, *J*=7.0 Hz), 7.79 (2H, m), 8.18 (1H, d, *J*=6.8 Hz), 9.37 (1H, s), 9.60 (1H, dd, *J*=9.2, 2.0 Hz). ¹³C-NMR δ: 22.6, 34.1, 41.1, 122.3, 126.2, 126.7, 127.3, 129.3, 130.7, 133.7, 141.2, 142.3, 144.1, 154.4, 159.1. Anal. Calcd for C₁₅H₁₂N₂Cl₂: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.76; H, 4.35; N, 9.51.

3-(2-Chloroethyl)-4-chloro-2-methyl-1,7-phenanthroline (12) This compound was obtained in 61% yield from **7** according to the general procedure used for the synthesis of **11**, mp 146–148 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M⁺, 46), 241 (96), 206 (32). ¹H-NMR (CDCl₃) δ: 2.85 (3H, s), 3.41 (2H, t, *J*=7.0 Hz), 3.62 (2H, t, *J*=7.0 Hz), 7.52 (2H, dd, *J*=12.0, 4.7 Hz), 8.06 (1H, d, *J*=10.0 Hz), 8.20 (1H, d, *J*=10.0 Hz), 9.01 (1H, d, *J*=2.9 Hz), 9.42 (1H, d, *J*=8.5 Hz). ¹³C-NMR δ: 24.5, 33.7, 41.6, 122.0, 122.7, 124.9, 128.7, 128.8, 129.2, 133.0, 142.2, 144.5, 149.22, 154.6, 158.0. Anal. Calcd for C₁₅H₁₂N₂Cl₂: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.99; H, 4.22; N, 9.56.

1-Chloro-2-(2-chloroethyl)-3-methyl-4,7-phenanthroline (13) This compound was obtained in 69% yield from **8** according to the general procedure used for the synthesis of **11**; mp 120–122 °C. (Recrystallization solvent ether–methanol 85/15). MS (relative intensity) *m/z*: 290 (M⁺, 37), 241 (94), 206 (23). ¹H-NMR (CDCl₃) δ: 2.66 (3H, s), 3.37 (2H, t, *J*=7.0 Hz), 3.63 (2H, t, *J*=7.0 Hz), 7.35 (2H, dd, *J*=4.5, 3.9 Hz), 7.88 (1H, d, *J*=9.0 Hz), 7.93 (1H, d, *J*=9.0 Hz), 8.70 (1H, d, *J*=8.0 Hz), 9.75 (1H, d, *J*=9.0 Hz). ¹³C-NMR δ: 24.1, 33.8, 41.2, 120.4, 124.2, 124.5, 124.5, 131.7, 132.9, 134.4, 141.5, 147.7, 148.2, 149.4, 158.0. Anal. Calcd for C₁₅H₁₂N₂Cl₂: C, 61.87; H, 4.15; N, 9.62. Found: C, 62.01; H, 4.08; N, 9.69.

4-Chloro-3-(2-chloroethyl)-2-methyl-1,10-phenanthroline (14) This compound was obtained in 54% yield from **9** according to the general procedure used for the synthesis of **11**, mp 204–206 °C. (Recrystallization solvent hexane–dichloromethane 85/15). MS (relative intensity) *m/z*: 291 (M⁺, 38), 290 (50), 241 (97). ¹H-NMR (CDCl₃) δ: 3.05 (3H, s), 3.55 (2H, t, *J*=7.0 Hz), 3.92 (2H, t, *J*=7.0 Hz), 7.74 (1H, dd, *J*=12.3, 4.3 Hz), 7.84 (1H, d, *J*=9.3 Hz), 8.19 (1H, d, *J*=9.3 Hz), 8.22 (1H, m), 9.19 (1H, d, *J*=2.6 Hz).

^{13}C -NMR δ : 24.5, 33.5, 41.1, 122.1, 123.4, 125.0, 126.5, 128.1, 130.3, 135.7, 142.1, 144.7, 145.1, 150.4, 160.2. *Anal.* Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_2\text{Cl}_2$: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.99; H, 4.23; N, 9.76.

4-Chloro-3-(2-chloroethyl)-2-methyl-1,8-phenanthroline (15) This compound was obtained in 63% yield from **10** according to the general procedure used for the synthesis of **11**; mp 148–150 °C. (Recrystallization solvent ether–ethyl acetate 70/30). MS (relative intensity) m/z : 290 (M^+ , 48), 241 (94), 206 (29). ^1H -NMR (CDCl_3) δ : 2.86 (3H, s), 3.52 (2H, t, $J=7.0$ Hz), 3.76 (2H, t, $J=7.0$ Hz), 7.77 (1H, d, $J=9.0$ Hz), 8.03 (1H, d, $J=9.0$ Hz), 8.74 (1H, d, $J=5.5$ Hz), 8.92 (1H, d, $J=5.5$ Hz), 9.24 (1H, s). ^{13}C -NMR δ : 24.4, 33.7, 41.4, 117.4, 122.7, 124.8, 125.9, 127.9, 130.0, 135.0, 142.1, 143.5, 145.7, 151.0, 158.1. *Anal.* Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_2\text{Cl}_2$: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.69; H, 4.31; N, 9.69.

4-Chloro-3-(2-chloroethyl)-2,10-dimethyl-1,10-phenanthroline Iodide (16) A solution of 2-methyl-3-(1'-chloroethyl)-4-chloropyrido[2,3-*i*]quinoline (**14**) (0.29 g, 1 mmol) and methyl iodide (5 mmol) in acetone (15 ml) was refluxed for 12 h. The resulting mixture was then cooled. The precipitate which formed was filtered, and washed with acetone to give 0.35 g (80%) of **16**; mp 186–188 °C. (Recrystallization solvent dichloromethane–ether 1/1). ^1H -NMR (CDCl_3) δ : 2.86 (3H, s), 3.48 (2H, t, $J=7.0$ Hz), 3.70 (2H, t, $J=7.0$ Hz), 5.31 (3H, s), 8.14 (1H, d, $J=9.0$ Hz), 8.16 (1H, dd, $J=10.0, 3.0$ Hz), 8.45 (1H, d, $J=9.0$ Hz), 9.14 (1H, d, $J=5.0$ Hz), 9.66 (1H, d, $J=4.5$ Hz). ^{13}C -NMR δ : 24.5, 33.2, 41.1, 55.1, 124.8, 126.7, 126.9, 128.7, 132.1, 135.7, 136.8, 139.2, 143.0, 146.8, 151.7, 159.1. *Anal.* Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_2\text{Cl}_2\text{I}$: C, 44.45; H, 3.50; N, 6.48. Found: C, 44.23; H, 3.37; N, 6.29.

4-Chloro-3-(2-chloroethyl)-2-methyl-1,10-phenanthroline Chloride (17) This product was obtained after bubbling HCl gas through a solution of **14** in ethanol until the hydrochloride salt of **14** was precipitated out of the solution; yield 95%, mp >260 °C. ^1H -NMR (CDCl_3) δ : 2.75 (3H, s), 3.37 (2H, t, $J=7.0$ Hz), 3.81 (2H, t, $J=7.0$ Hz), 7.71–8.07 (3H, m), 8.73 (1H, d, $J=8.6$ Hz), 8.88 (1H, d, $J=5.2$ Hz).

Plasmodium falciparum Growth Inhibition Assay Three strains of *P. falciparum* were used: the chloroquine resistant FcB1-Columbia and FcM29 strains, and a Nigerian chloroquine-sensitive strain.¹¹ Parasites were cultured according to the method originally described by Trager and Jensen.⁸ Culture medium was replaced daily and the cultures were synchronized every 48 h by 5% D-sorbitol lysis (Merck, Darmstadt, Germany).¹² The method used for *in vitro* antimalarial activity testing was adapted from the radioactive micromethod of Desjardins *et al.*¹³ The molecules were tested 3 times in triplicate in 96-well plates (TPP, Switzerland) with cultures at ring stage at 0.5–1% parasitaemia (hematocrit: 1%). For each test, the parasite cultures were incubated with the chemicals at decreasing concentrations for 24 and 72 h. The first dilution of the product (10 mg/ml) was performed with dimethylsulfoxide (DMSO, Merck), and the following with RPMI 1640. Parasite growth was estimated by [^3H]-hypoxanthine (Amersham-France, Les Ulis, France) incorporation.¹¹ The parasite [^3H]-hypoxanthine incorporation in the presence of chemicals was compared with that of control cultures without chemicals (mean of the corresponding wells was referred to as

100%) or with DMSO. Concentrations inhibiting 50% of the parasite [^3H]-hypoxanthine incorporation (IC_{50}) were determined graphically in concentration *versus* percent inhibition curves. Chloroquine (diphosphate, Sigma) sensitivity was checked every month: the IC_{50} value for the Nigerian chloroquine-sensitive strain was 41 (<70 nM) and for the chloroquine-resistant FcB1-Columbia and FcM29 strains, the IC_{50} were 145 nM and 210 nM respectively (>100 nM).¹⁴ Toxicity of the chemicals was estimated on human fibroblasts (HeLa). The cell line was cultured under the same conditions as *P. falciparum*, except for 5% human serum which was replaced by 5% fetal calf serum (Boehringer). Subcultures were obtained after treatment with Trypsine (0.125%) EDTA (0.02%) in PBS (Gibco). For determination of the cytotoxicity, cells were distributed in 96-well plates at 2×10^3 cells/well in 100 μl , then 100 μl of culture medium containing chemicals at various concentrations were added. Cell growth was estimated by [^3H]-hypoxanthine (Amersham) incorporation after 24 and 72 h incubations. The [^3H]-hypoxanthine incorporation in the presence of drugs was compared with that of control cultures without chemicals.¹¹

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