Synthesis, Antimalarial Activity, and Molecular Modeling of New Pyrrolo[1,2-*a*]quinoxalines, Bispyrrolo[1,2-*a*]quinoxalines, Bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines, and Bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines

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Received November 3, 2003

Three pyrrolo[1,2-*a*]quinoxalines, 15 bispyrrolo[1,2-*a*]quinoxalines, bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines, and bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines were synthesized from various substituted nitroanilines or nitropyridines and tested for their in vitro activity upon the erythrocytic development of *Plasmodium falciparum* strains with different chloroquine-resistance status. Bispyrrolo[1,2-*a*]quinoxalines showed superior antimalarial activity with respect to monopyrrolo[1,2-*a*]quinoxalines. The best activity was observed with bispyrrolo[1,2-*a*]quinoxalines linked by a bis(3-aminopropyl)piperazine. Moreover, it was observed that the presence of a methoxy group on the pyrrolo[1,2-*a*]quinoxaline nucleus increased the pharmacological activity. Drug effects upon β-hematin formation were assayed and showed similar or higher inhibitory activities than CQ. A possible mechanism of interaction implicating binding of pyrroloquinoxalines to β-hematin was supported by molecular modeling.

Introduction

It is estimated that there are 300−500 million cases of malaria annually resulting in 1−3 million deaths, most of which are children under the age of 5. Malaria is a particularly important disease in sub-Saharan Africa, where about 90% of cases and deaths occur but is also a serious public health problem in certain regions of southeast Asia and South America.[1,2] Human malaria is caused by four species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*), which are transmitted by female *Anopheles* mosquitoes. The majority of cases of malaria and deaths are caused by *P. falciparum*. Present chemotherapies are proving to be inadequate, are toxic, or are becoming ineffective because of an increase in resistance.[3−5] Chloroquine (CQ)[6] is, after 50 years, still a mainstream drug in the fight against malaria, but its efficacy is being steadily eroded by the development of resistant parasites.[5,7] CQ is believed to exert its activity by inhibiting hemozoin formation in the digestive vacuole of the malaria parasite,[8,9] though this has been recently questioned by Ginsburg and coworkers who have suggested that inhibition of the degradation of ferriprotoporphyrin IX by glutathione-dependent redox processes could be a second mode of action of CQ.[10] Biochemical studies indicate that isolates of the CQ-resistant parasite accumulate less drug in the food vacuole than their more sensitive counterparts. However, opinion remains divided on the mechanistic explanations for the reduction: (1) a rapid CQ efflux mechanism by CQ-resistant parasites,[11] (2) an elevated pH in the food vacuole of the CQ-resistant parasites,[12] (3) resistance being linked to a carrier-mediated CQ uptake,[13] (4) resistance being linked to a reduced CQ affinity to ferriprotoporphyrin.[14] Concerning the first explanation, its reversal by molecules such as verapamil, desipramine, and chlorpromazine suggests that an enhanced CQ efflux by a multidrug-resistant mechanism may be implicated.[11−15] A possibility of overcoming this multidrug-resistant mechanism is to design new quinoline-based drugs that will not be recognized by the protein system involved in drug efflux. In this regard, bulky bisquinoline antimalarial drugs are suggested to be extruded with difficulty by a proteinaceous transporter.[16] Previous toxicity results have inhibited the development of Ro 47-7737,[17] and then further linker modulations led to the promising **A** model of bisquinolines.[16,18−23] These compounds show much lower resistance indices than CQ, suggesting that the bisquinoline structure is less efficiently excluded by drug-resistant

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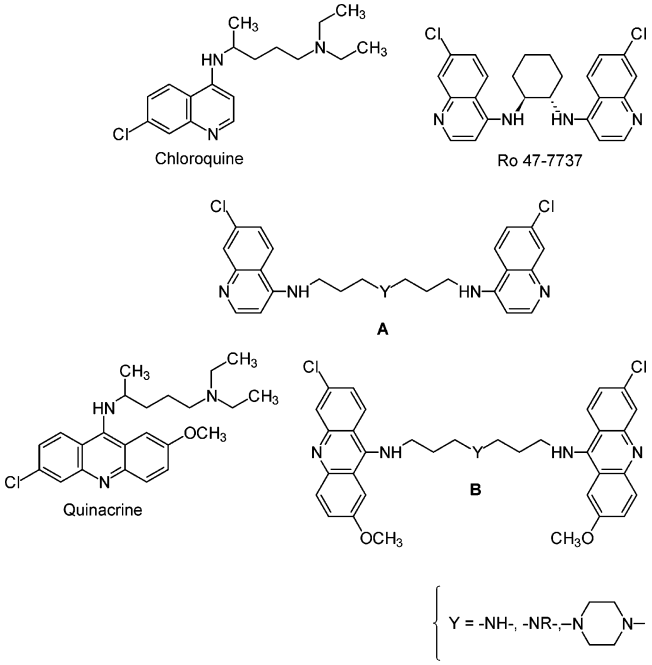
10.1021/jm0310840 CCC: $27.50
Published on Web 03/16/2004

Chart 1. Structure of Chloroquine, Ro 47-7737, Bisquinolines **A**, Quinacrine, and Bisacridines **B**



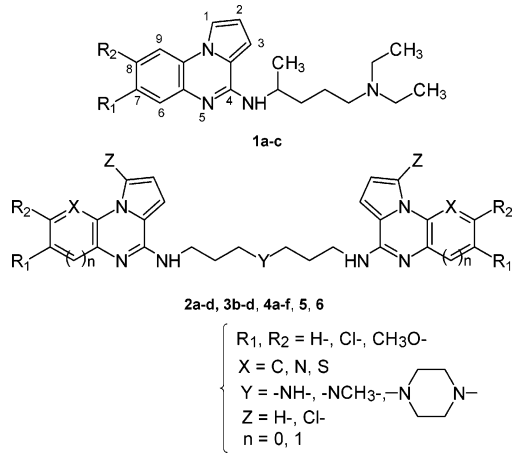
parasites. Moreover, the bisquinolines drugs were proved to be active against CQ-resistant malaria, with a longer duration of action and lower toxicity. On the other hand, acridine derivatives have also been considered as potential antimalarial drugs. For instance, quinacrine,[24] the 9-amino-6-chloro-2-methoxyacridine analogue of CQ, was used clinically before CQ.

Recently, bisacridines **B** with a symmetrical linker $-NH-(CH_2)_3-Y-(CH_2)_3-NH-$ (Y = $-NH-$, $-NR-$, or piperazine) were described as potent antimalarial drugs (Chart 1).[25,26]

We previously described a novel synthetic approach to pyrrolo[1,2-*a*]quinoxaline derivatives as interesting bioactive analogues of quinoline or quinoxaline compounds.[27−30] Hence, the pyrrolo[1,2-*a*]quinoxaline moiety could be developed as a template for the design of bisheterocyclic bioactive compounds. We report here the synthesis and in vitro antiparasitic activity study upon *Plasmodium falciparum* of a series of pyrrolo[1,2-*a*]-quinoxalines **1** and of bispyrrolo[1,2-*a*]quinoxalines, bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines **2**−**5**, and bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines **6** in which aromatic nuclei are joined by aliphatic polyamines linker (Chart 2). The choice of the linker was based on previous results obtained for bisquinolines and bisacridines. All these new derivatives have in common a bis- or tricyclic aromatic moiety with the reference compounds but joined by a bis(3-aminopropyl)amine linker via a "pseudo-amidinic" bond. To examine the mechanism of action of these bispyrrolo[1,2-*a*]quinoxalines, we have set up an in vitro assay of β-hematin formation under conditions that are designed to resemble the conditions within the food vacuole. The lipophilicity behavior dependence of new compounds was studied with respect to the parasite vacuolar and cytosolic pHs.

Finally, a molecular modeling study was carried out to elucidate the mechanism of action implicating binding

Chart 2. Structure of Synthesized Pyrrolo[1,2-*a*]quinoxalines **1a**−**c**, Bispyrrolo[1,2-*a*]quinoxalines **2a**−**d**, **4a**−**d**, and **5**, Bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines **4e**,**f**, and Bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines **6**



of pyrroloquinoxalines to β-hematin by using X-ray crystal structure results obtained for compounds **4b** and **4e**.

Chemistry

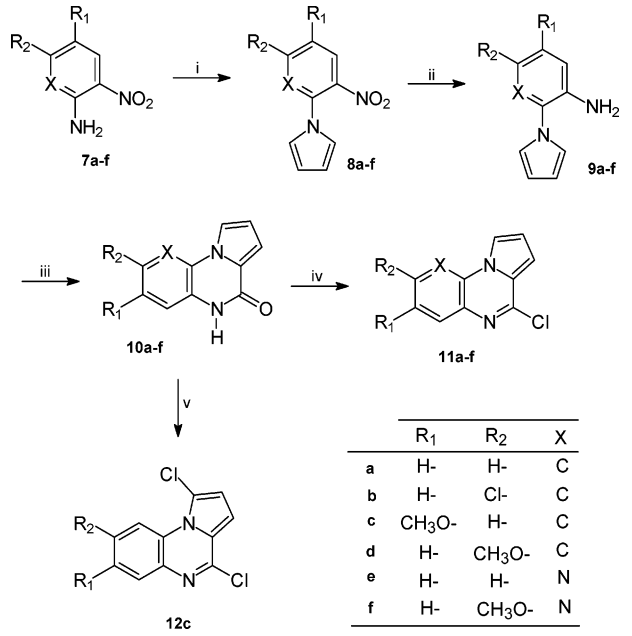
The new pyrrolo[1,2-*a*]quinoxalines and pyrido-[3,2-*e*]pyrrolo[1,2-*a*]pyrazines **1a**−**c**, **2a**−**d**, **3b**−**d**, **4a**−**f**, and **5** or pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines **6** were synthesized from substituted 2-nitroanilines or pyridines **7a**−**f**.[28−30] Not commercialy available 5-methoxy-2-nitroaniline **7d** and 2-amino-6-methoxy-3-nitropyridine **7f** were prepared according to the literature.[30−32] The Clauson−Kaas reaction of anilines **7a**−**f** with 2,5-dimethoxytetrahydrofuran (DMTHF) in acetic acid gave the pyrrolic derivatives **8a**−**f** in 75−81% yields, which were then reduced using a $BiCl_3-NaBH_4$ treatment to provide the attempted 1-(2-aminophenyl)pyrroles **9a**−**f** (Scheme 1).

The cyclization was then possible between the NH_2 and the C-α of the pyrrole ring by reacting **9a**−**f** with triphosgene in toluene to give the lactams **10a**−**f**, which were subsequently chlorodehydroxylated with phosphorus oxychloride to obtain the chloroquinoxalines **11a**−**d** or the chloropyrazines **11e**−**f**.[28] Reaction of **10c** with a $POCl_3-PCl_5$ mixture led to the dichloroderivative **12c**, as previously described.[33] The placement of the second chlorine atom at position 1 was suggested by the AB system due to H-2 and H-3 protons. Displacement of the chlorine atom of **11a**−**c** with 5-diethylamino-2-pentylamine led to the pyrrolo[1,2-*a*]quinoxalines **1a**−**c** (58−79% yields), structural analogues of 2-aminoquinolines (Scheme 2).

The bispyrrolo[1,2-*a*]quinoxalines and bispyrido-[3,2-*e*]pyrrolo[1,2-*a*]pyrazines **2a**−**d**, **3b**−**d**, **4a**−**f**, and **5** were synthesized by reacting 3,3′-diamino-*N*-methyldipropylamine or *N*-(3-aminopropyl)-1,3-propanediamine or 1,4-bis(3-aminopropyl)piperazine) with 2 equiv of **11a**−**f** or **12c** in DMF at 130 °C in the presence of potassium carbonate as an inorganic base (Schemes 3 and 4).

A similar nucleophilic substitution with 1,4-bis(3-aminopropyl)piperazine and 2 equiv of 5-chloropyrrolo-

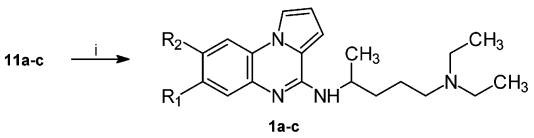
Scheme 1[a]



	R_1	R_2	X
a	H-	H-	C
b	H-	Cl-	C
c	CH_3O-	H-	C
d	H-	CH_3O-	C
e	H-	H-	N
f	H-	CH_3O-	N

[a] (i) DMTHF, Δ; (ii) $BiCl_3$, $NaBH_4$, EtOH; (iii) $CO(OCCl_3)_2$, toluene, Δ; (iv) $POCl_3$, Δ; (v) $POCl_3$, PCl_5, Δ.

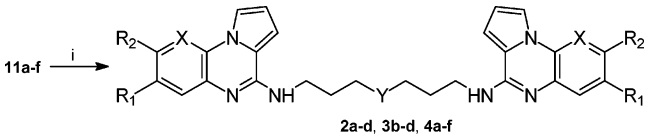
Scheme 2[a]



	R_1	R_2
a	H-	H-
b	H-	Cl-
c	CH_3O-	H-

[a] (i) 5-Diethylamino-2-pentylamine, Δ.

Scheme 3[a]



	R_1	R_2	X
a	H-	H-	C
b	H-	Cl-	C
c	CH_3O-	H-	C
d	H-	CH_3O-	C
e	H-	H-	N
f	H-	CH_3O-	N

	Y
2a-d	-NCH_3-
3b-d	-NH-
4a-f	-N N-

[a] (i) $H_2N-(CH_2)_3-Y-(CH_2)_3-NH_2$, K_2CO_3, DMF, Δ.

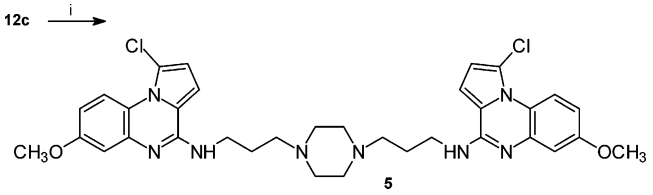
[1,2-*a*]thieno[3,2-*e*]pyrazine **13**[34] furnished the bispyrrolo-[1,2-*a*]thieno[3,2-*e*]pyrazines **6** (Scheme 5).

Compounds **1a**−**c**, **2a**−**d**, **3b**−**d**, **4a**−**f**, **5**, and **6** were then converted into their oxalate salts in 32−80% yields by treatment with oxalic acid in refluxing 2-propanol (Table 1).

Pharmacology

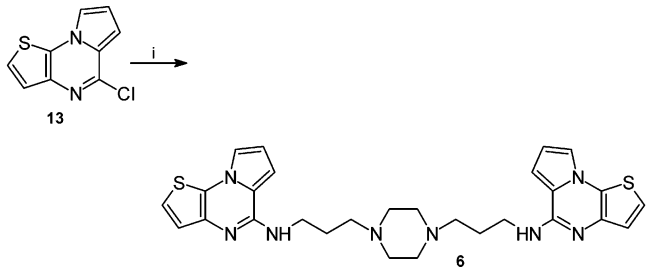
Antimalarial Activity. All compounds were tested for their antimalarial activity in vitro upon the *P.*

Scheme 4[a]



[a] (i) $H_2N-(CH_2)_3-$piperazine$-(CH_2)_3-NH_2$, K_2CO_3, DMF, Δ.

Scheme 5[a]



[a] (i) $H_2N-(CH_2)_3-$piperazine$-(CH_2)_3-NH_2$, K_2CO_3, DMF, Δ.

Table 1. Physical Properties of the Final Amines (**1**−**6**)

compd	salt[a]	mp (°C)[b]	% yield[c]
1a	2 oxalate	71−72	46
1b	2 oxalate	73−75	48
1c	2 oxalate	72−74	51
2a	3 oxalate	144−146	39
2b	3 oxalate	210−211	62
2c	3 oxalate	125−126	49
2d	3 oxalate	87−89	55
3b	3 oxalate	158−159	70
3c	3 oxalate	108−110	33
3d	3 oxalate	186−188	31
4a	4 oxalate	203−204	58
4b	4 oxalate	209−211	57
4c	4 oxalate	221−223	47
4d	4 oxalate	197−199	32
4e	4 oxalate	244−246	80
4f	4 oxalate	231−233	61
5c	4 oxalate	187−189	72
6	4 oxalate	210−212	28

[a] Amines **1**−**6** were dissolved in 30 mL of 2-propanol, heated to boiling, and treated with oxalic acid (4 equiv, based on the amount of the starting material). The oxalate salts crystallized upon cooling, were collected by filtration, and were washed with 2-propanol and Et_2O. [b] Crystallization solvent: 2-PrOH−H_2O. [c] The yields included the conversions into the oxalates.

falciparum CQ-sensitive strain Thai (IC_{50}(CQ) = 0.01 μM) and the CQ-resistant strains FcB1 and K1 (IC_{50}-(CQ) = 0.13 and 0.24 μM, respectively). As shown in Table 2, bispyrrolo[1,2-*a*]quinoxalines **2**−**6** were always more active (IC_{50} values in the nanomolar range) than their respective monomers **1a**−**c** (IC_{50} values in the micromolar range). For a same substitution on the pyrroloquinoxaline moiety, compounds joined by a bis-(3-aminopropyl)piperazine linker (compounds **4**−**6**) were generally more active (up to 3 times) than their counterparts with bis(3-aminopropyl)methylamine (**2**) or bis-(3-aminopropyl)amine linkages (**3**), with the exception of **2c** and **4c**, joined by a bis(3-aminopropyl)methylamine and a bis-(3-aminopropyl)piperazine linker respectively, which showed similar antimalarial activities. These new derivatives have a bis- or tricyclic aromatic moiety in common with the reference compounds, but they are linked to the bis(3-aminopropyl)amine linker via a "pseudoamidinic" bond. Compounds **2** and **3**

Table 2. In Vitro Sensitivity of *P. falciparum* Strains and in Vitro Cytotoxicity on Mammalian L6 Cells

	IC_{50} values (μM)[a]						
	P. falciparum strains			mammalian L6 cells	index of selectivity[b]	resistance index	
compd	Thai	FcB1	K1			FcB1[d]	K1[e]
CQ	0.01 ± 0.01	0.13 ± 0.04	0.24 ± 0.03	ND[c]	ND[c]	13	24
1a	2.95 ± 0.13	4.97 ± 0.41	4.34 ± 0.27	>20	>4.6	1.7	1.5
1b	1.94 ± 0.65	4.79 ± 0.09	3.06 ± 0.44	17.31 ± 0.48	5.6	2.5	1.6
1c	0.83 ± 0.04	2.47 ± 0.17	1.78 ± 0.25	>20	>11.2	3.0	2.1
2a	0.12 ± 0.01	0.48 ± 0.01	0.28 ± 0.05	6.2 ± 0.1	22.1	4.0	2.3
2b	0.40 ± 0.12	1.01 ± 0.27	0.70 ± 0.01	4.95 ± 0.96	7.1	2.5	1.8
2c	0.05 ± 0.01	0.11 ± 0.01	0.05 ± 0.01	5.74 ± 0.15	106.3	2.2	1.0
2d	0.12 ± 0.03	0.32 ± 0.03	0.20 ± 0.01	3.79 ± 0.63	19.2	2.7	1.7
3b	0.47 ± 0.05	1.14 ± 0.06	0.79 ± 0.04	4.81 ± 1.05	6.1	2.4	1.7
3c	0.04 ± 0.01	0.19 ± 0.01	0.11 ± 0.02	2.98 ± 0.06	26.6	4.7	2.7
3d	0.14 ± 0.02	0.30 ± 0.04	0.33 ±0.03	3.62 ± 0.62	11	2.1	2.4
4a	0.08 ± 0.01	0.29 ± 0.02	0.37 ± 0.15	5.04 ± 0.19	13.6	3.6	4.6
4b	0.28 ± 0.05	0.64 ± 0.04	0.48 ± 0.09	4.01 ± 0.66	8.2	2.3	1.7
4c	0.03 ± 0.01	0.13 ± 0.01	0.08 ± 0.02	1.32 ± 0.09	17.4	4.3	2.7
4d	0.04 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	4.06 ± 0.72	52.7	3.2	2.0
4e	ND[c]	1.09 ± 0.10	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]
4f	ND[c]	0.60 ± 0.02	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]
5	0.25 ± 0.05	0.72 ± 0.03	0.33 ± 0.01	3.07 ± 0.69	9.3	2.9	1.32
6	ND[c]	0.93 ± 0.03	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]

[a] IC_{50} values were measured on the chloroquine-sensitive strain Thai/Thailand and the chloroquine-resistant strains FcB1/Colombia and K1/Thailand. The IC_{50} values correspond to the mean ± standard deviation from three independent experiments. [b] Index of selectivity is defined as the ratio of the IC_{50} value on the mammalian L6 cells to the IC_{50} value against the *P. falciparum* K1 strain. [c] ND: not determined. [d] IC_{50}(FcB1)/IC_{50}(Thai). [e] IC_{50}(K1)/IC_{50}(Thai).

bearing bis(3-aminopropyl)methylamine and an amine linkage generally exhibit similar activities. Decrease of the lipophilicity of molecules by hydroxymethylation either on carbon C-7 (compounds **2c**, **3c**, and **4c**) or on carbon C-8 (compounds **2d**, **3d**, and **4d**) of the pyrroloquinoxaline moiety increased the antimalarial activity up to 5 times when compared to the nonsubstituted counterparts (compounds **2a**, **4a**). Finally, this hydroxymethyl substitution achieved on C-7 gave the most active compounds **2c**−**4c**.

In contrast, the addition of a chlorine atom on the pyrroloquinoxaline moiety, in analogy with CQ and with **B**, reduced the activity up to 10 times (i.e., **4a** compared to **4b**, and **4c** compared to **5**). In the same way, replacement of the pyrroloquinoxaline moiety by a pyridopyrrolopyrazine or a pyrrolothienopyrazine moiety, bioisosteres of the pyrroloquinoxaline nucleus, reduced the antimalarial activity about 5 times (i.e., **4a** compared to **4e** and **6**), suggesting that introduction of an electron-rich (or electronegative) atom, such as a nitrogen or sulfur atom, decreases this activity. On the other hand, introduction of a methoxy substituent on position C-2 of the pyridopyrrolopyrazine moiety (compound **4f**) seems to increase the activity about 2 times in comparison with derivative **4e**, corroborating the results obtained with **4c** in comparison with **4a**.

As seen in Table 2, almost all compounds have lower IC_{50} values for inhibition of growth of CQ-sensitive strain Thai than for inhibition of growth of CQ-resistant strains FcB1 and K1. A comparison of the IC_{50} values for the inhibition of growth of the resistant and sensitive strains of *P. falciparum* suggests relatively low levels of cross-resistance to CQ. The resistance index values calculated from the comparison of the IC_{50} values of the resistant and sensitive strains of *P. falciparum* were lower than those for CQ, in the ranges 1.7−4.7 (FcB1) and 1.0−4.6 (K1) (Table 2). Because it was already reported for bisquinolines, these results could be discussed in terms of recognition difficulties related to the bulky structure of studied bispyrroloquinoxalines by the efflux proteins involved in conferring CQ resistance.[16,20]

Cytotoxicity. All tested bispyrrolo[1,2-*a*]quinoxalines showed cytotoxicity upon the mammalian L6 cells in the micromolar range, and the different substitutions had less influence on the IC_{50} values than that observed for the antimalarial activity; e.g., for the piperazine series where marked differences of cytotoxicity were observed, IC_{50} values on the L6 cells varied from 1.3 to 5.0 μM (Table 2). Index of selectivity was defined as the ratio of the IC_{50} value on the mammalian cells to the IC_{50} value on the CQ-resistant *P. falciparum* strain K1. Compounds that demonstrated high selectivity (high index of selectivity) should offer the potential of safer therapy. This led to the identification of compounds with index of selectivity greater than 50 for compound **4d** and greater than 100 for compound **2c** that could constitute suitable candidates for further pharmacological studies.

Inhibition of β-Hematin Formation. In vitro experiments have previously established that quinoline antimalarial drugs such as CQ are associated with the crystallization of haemazoin.[35−38] In fact, current evidence indicates that drugs act by inhibiting the formation of haemazoin and thus preventing haem detoxification. Three mechanisms of action can be proposed: (1) direct binding of the drug to haem monomers or dimers in solution, which interferes with the crystallization of haemazoin;[37] (2) enzymatic inhibition of a protein that catalyzes haemazoin crystallization;[39] and (3) chemiabsorption of the drug onto crystallized haemazoin, leading to inhibition of further haem aggregation.[40]

Given that methoxy substitution on C-7 resulted in the most active compounds in precedent tests, the three 7-methoxy derivatives **2c**, **3c**, and **4c** (only differing by the nature of the linker) were tested and compared to CQ in the ability to inhibit β-hematin formation (Figure 1), the main mechanism of action of CQ, according to two selected protocols.[8,9,35−37] By use of insoluble para-

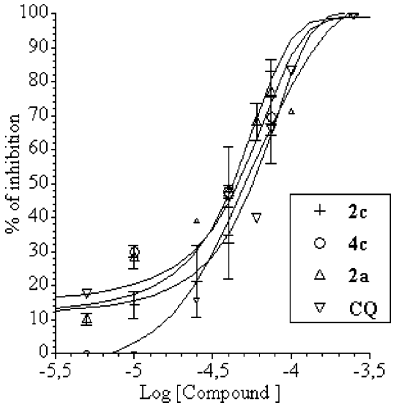


Figure 1. Percent inhibition of β-hematin formation versus log(concentration of compound) for CQ and compounds **2a**, **2c**, and **4c**.

Table 3. Vacuolar Accumulation Ratios (VARs) and log *D* Values for Some Selected Compounds at pH 7.4 and pH 5

compd	VAR ($\times 10^3$)	log $D_{7.4}$	log D_5
CQ	61.1	ND[a]	ND[a]
2a	225.6	4.12	1.56
2b	1.8	4.44	3.5
2c	105.4	3.55	0.93
2d	42.1	3.41	0.41
3b	1.6	5.06	3.16
3c	104.2	2.76	0.8
3d	41.7	2.58	0.93
4a	409.1	2.52	1.31
4b	2.1	4.92	3.43
4c	186.9	3.72	2.15
4d	72.5	4.67	1.88
4e	0.1	5.05	2.22
4f	33.0	4.73	3.97
5	0.9	5.74	4.17

[a] ND = not determined.

site material as catalyst,[41] compounds **2c**, **3c**, and **4c** at 100 μM inhibit 89%, 100%, and 59% β-hematin formation, respectively. At the same concentration, 82% of inhibition was measured for CQ. By use of 1-monooleoyl glycerol as catalyst,[42,43] compounds **2a**, **2c**, **3c**, **4c**, and CQ showed a β-hematin formation inhibition with IC_{50} values of 51, 60.5, 75, 47.9, and 59.9 μM, respectively (Figure 1), while the IC_{50} values for **2b**, **4a**, and **4b** were found to be superior at 75, 100 and 100 μM, respectively. These data could support the hypothesis that bispyrroloquinoxalines inhibit parasite growth via inhibition of β-hematin formation.

However, this approach also requires that these symmetrical triprotic or tetraprotic bispyrroloquinoxalines, like the diprotic weak base CQ, concentrate to micromolar levels in the acidic food vacuole of the parasite, the organelle in which β-hematin formation takes place.[44]

The preliminary pharmacological results could be discussed in terms of physicochemical behavior through the partitioning theory,[4] which involved the influence of pK_a and lipophilicity of studied compounds. Hence, these two parameters determine the absorption, permeability, and in vivo distribution of drugs. Consequently, to evaluate the contribution to antimalarial activity of the component accumulation in the food vacuole, HPLC determination of log *D* at vacuolar and cytosolic pHs (5.0 and 7.4, respectively)[45] and in silico vacuolar accumulation ratios (VAR) based on a weak-base model were carried out for the 14 bispyrrolo[1,2-*a*]quinoxalines **2a**−**d**, **3b**−**d**, **4a**−**f**, and **5**.[46]

As shown in Table 3, methoxy-substituted compounds **2d**−**4d** and **2c**−**4c** would accumulate 30−70 times more than the corresponding 8-chloro-substituted compounds **2b**−**4b**, which correlates with an increase of the in vitro antimalarial activity. For example, compound **4c** (VAR = 186.9×10^3, log $D_5 = 2.15$, log $D_{7.4} = 3.72$) displayed a greater accumulation than compound **4b** (VAR = 2.1×10^3, log $D_5 = 3.43$, log $D_{7.4} = 4.92$) and is about 5-fold more active. Hence, the introduction of a methoxy substituent seems to considerably increase ion-trapping of molecules. The best antimalarial activities were obtained for the methoxy-substituted compounds **2c**,**d** and **3c**,**d** displaying predicted VAR values in the range of 41.7×10^3 to 186.9×10^3. Replacement of the methoxy group in compounds **c** and **d** by a hydrogen atom (**a**) increases the predicted VAR values (more than 2- to 5-fold), while the biological activity was twice less important. On the other hand, for all compounds there is a small difference in the range of lipophilic values at pH 7.4, whereas there appears to be a larger difference (more than 10-fold) at pH 5.0. The log *D* values of the most active compounds **c**−**d**, determined at pH 5.0, range between 0.41 and 0.93 for series **2** and **3** and between 1.88 and 2.15 for series **4**. Replacement of the methoxy function with hydrogen has little effect on the lipophilicity, whereas introduction of a chlorine group increases it.

These preliminary results could lead to the hypothesis that in addition to some specific mechanism of action, the permeation of the tested compounds is certainly implicated in their biological activity and also supports that both pH trapping and β-hematin inhibition could be some basis of antiplasmodial activity of these new bisquinoline analogues.

Molecular Modeling. Recently, Buller et al.[37] have described a theoretical growth of synthetic hemozoin and proposed a noncovalent binding site for the quinoline drug family at the end face of the fastest-growing direction of synthetic hemozoin. Pagola[47] has proposed that the antimalarial quinolines act through growth inhibition of hemozoin crystals as a result of absorption onto its actively growing faces. With these studies in mind and in order to evaluate the implication of spatial conformation of synthesized bispyrroloquinoxalines, we carried out a molecular modeling study based on the crystallographic analysis of compounds **4b** and **4e**.

As shown in Figure 2,[48] the pyrrolo[1,2-*a*]quinoxaline ring systems are planar and are parallel to each other, related by a center of symmetry. In the crystal, the distances between the pyrrolo[1,2-*a*]quinoxaline rings of **4b** and **4e** bound with symmetrical linker −NH−$(CH_2)_3$−piperazine−$(CH_2)_3$−NH− is observed at 9.919 and 10.208 Å, respectively. The piperazine rings have the chair conformation, and the atoms are alternately displaced from the least-squares plane by 0.69 Å for **4b** and by 0.68 Å for **4e**. In **4b** and **4e**, the distances between the least-squares planes of the two pyrroloquinoxaline moieties were approximatively 2.40 and 2.05 Å, respectively. By use of these experimental crystallographic data, energy-minimized molecular conformations were generated for the bisquinoline analogues and then were examined for their likely interaction with the synthetic hemozoin described by Buller et al.[37]

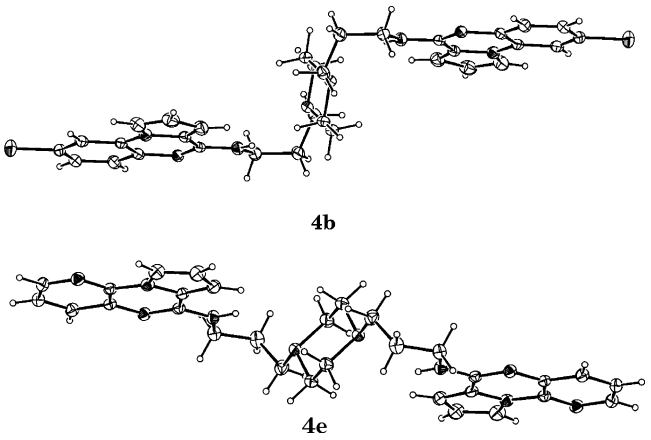


Figure 2. Side views of the crystal structures of **4b** and **4e**. Displacement ellipsoids are drawn at the 30% probability level.

The "quinoline-like" antimalarial compounds are accumulated in the food vacuole of the parasite (pH 5.0), so modeling studies were concentrated on the tetraprotonated forms of the drugs. Simulation studies were carried out on compounds **4b** and **4d**. Each molecule was energy-minimized from the "as-constructed" conformation using the extensible systematic force field (ESFF),[49] the Discover program,[50] and Gamess.[51]

From this procedure, 10 molecular conformations were generated and three final structures were selected that are in agreement with the nonprotonated analogues from X-ray crystallography (Figure 2), for which the aromatic pyrrolo[1,2-*a*]quinoxaline ring systems are planar and parallel to each other (Figure 3). This result corroborated the employment of the ESFF method in this study.

The inter-nitrogen separation distances between the first protonated nitrogen of the piperazine and the first protonated nitrogen of the quinoxaline and between the second protonated nitrogen of the piperazine and the second protonated nitrogen of the quinoxaline are between 6.94−7.43 and 7.36−7.38 Å for **4b** and between 6.92−6.98 and 7.04−7.24 Å for **4d**. In all cases, it was observed that the inter-nitrogen atom separations increased on protonation of the studied molecules, contrary to the values found for X-ray crystallography (5.12 Å for **4b**).

After generation of a set of conformations for **4b** and **4d**, an investigation into their interactions with the synthetic hemozoin was carried out. A model of the hematin molecular unit linked through iron−carboxylate bonds to the centrosymmetric cyclic dimer was constructed (Figure 4). This unit was then energy-minimized with the ESFF potential set. Three MD runs at 300 K for a simulation time of 5 ps were performed, and the resulting structure was energy-minimized to gain a low-energy conformation. The three obtained molecular unit conformations are very closed and successfully reproduce the expected hematin molecular unit (Figure 4b).[37] Four molecular units (Figure 4a) were assemblied manually to obtain a "pseudo"-β-hematin with respect to the distances observed between the adjacent β-hematin in the crystal lattice (Figure 5).[47] The conformation and docking surface site position and orientation of the pyrroloquinoxalines **4b** and **4d** were adjusted manually in this tetramer to yield acceptable interatomic contact distances as described by Buller et al.[37] (Figures 6 and 7). This model is in keeping with NMR studies on the interaction between CQ and hematin.[52] CQ and analogues form an (aromatic)N−HC═C(vinyl) hydrogen bond, and a $CCl-H_3C$ interaction with the host molecule helps to anchor the guest within the crevice. It was proposed that aromatic interactions of the pyrroloquinoxalines **4b** and **4d** are similar to those of CQ and analogues (Figures 6 and 7). Indeed, the size of the aromatic moiety is critical for crystal inhibition,[36] but the pyrroloquinoxaline aromatic nucleus could be bound within the crevice as the quinoline aromatic ring.

In the case of CQ, the exocyclic chain must twist the C4−N(aniline) bond to form a salt bridge with the carboxylate moiety and allows intercalation of the aromatic moiety within the crevice. For studied compounds, it is possible to define salt bridges of protonated piperazine N atoms with the carboxylate moiety of hematin, while the pyrroloquinoxaline nucleus could destabilize the complex by a $\pi-\pi$ intercalation between two hematin molecular units (Figures 6 and 7).

Such a $\pi-\pi$ interaction of the pyrroloquinoxaline ring over the porphyrine could bring another noncovalent binding site for the bispyrroloquinoxaline drug family at the end face of the fastest-growing direction of synthetic haemozoin. Finally, pyrroloquinoxalines derivatives could also bind to and trap ferriprotoporphyrin in a μ-oxo dimeric form and prevent the formation of the β-hematin dimers that are required for haemozoin formation.[35,52,53]

Finally, this molecular modeling study should be considered as a preliminary hypothetical approach to enlighten the antimalarial activity mechanism of bispyrroloquinoxaline derivatives. Nevertheless, further and more elaborate modeling studies are required to confirm the defined drug binding site on malarial pigment crystal.

Conclusion

In the present report, we have described the synthesis and the antimalarial activity of new pyrrolo[1,2-*a*]-quinoxalines **1** and bispyrrolo[1,2-*a*]quinoxalines **2**−**6** in which aromatic nuclei are joined by aliphatic polyamines linker. Through this study, it was observed that bispyrroloquinoxalines **2**−**6** showed superior antimalarial activity with respect to the monopyrroloquinoxaline compounds, structural analogues of CQ. Introduction of a methoxy substituent on the pyrroloquinoxaline nucleus had an influence on the activity, in relation to the lipophilicity of described compounds. In the bisheterocycles, a piperazine moiety was the best linker to confer activity through a pseudosymmetry of the molecule.

A preliminary molecular modeling study was carried out to elucidate the action mechanism of **2**−**6** through the implication of the molecules binding to β-hematin crystal surface. On the basis of these findings, it is possible to further identify new pyrrolo[1,2-*a*]quinoxaline derivatives that inhibit β-hematin formation by semirational design.

Experimental Section

Chemistry. Commercial reagents were used as received without additional purification. Melting points were determinated with an SM-LUX-POL Leitz hot-stage microscope and

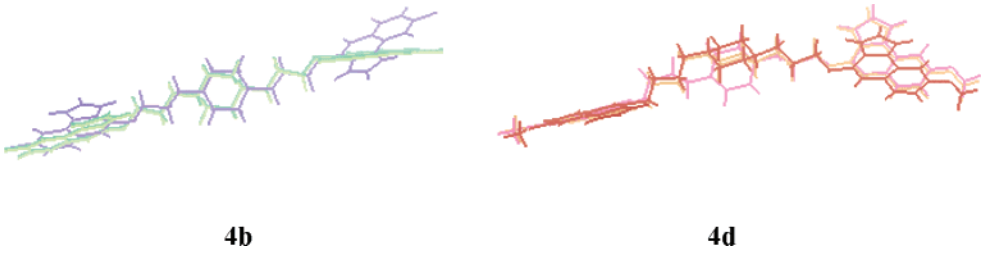


Figure 3. View of the backbone of the final selected superimposed structures.

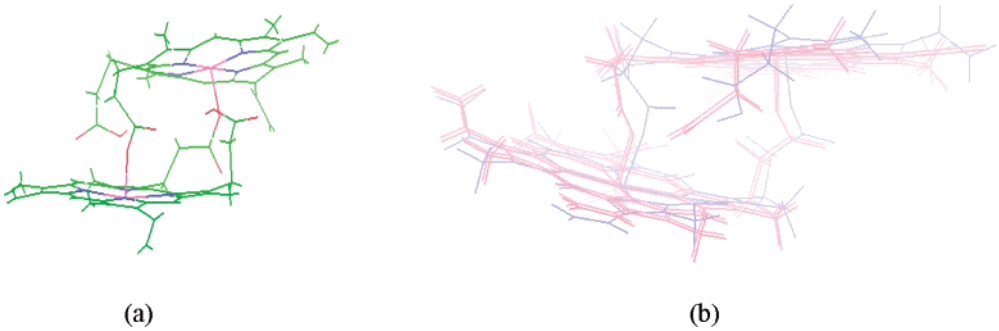


Figure 4. (a) Model of hematin molecular unit with propionate oxygen−iron bonds. (b) View of the backbone of the final selected superimposed structures.

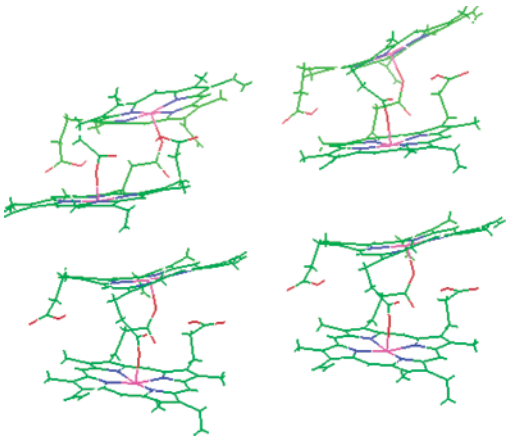


Figure 5. "Pseudo"-β-hematin.

are uncorrected. IR spectra were recorded on an BRUKER IFS-25 spectrophotometer. NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AC 200 spectrometer (^{1}H, ^{13}C, 2D-COSY). Splitting patterns have been designated as follows: s = singlet; bs = broad singlet; d = doublet; t = triplet; q = quartet; m = mutiplet. Analytical TLC was carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV$_{254}$) with visualization by irradiation with a UV lamp. Silica gel 60 (70−230 mesh) was used for column chromatography. Elemental analyses (C, H, N) for new compounds were performed by CNRS (Vernaison-France), and the results agreed with the proposed structures within ±0.3% of the theoretical values.

1-(5-Chloro-2-nitrophenyl)pyrrole (8b). A mixture of 2-nitroaniline **7b** (0.07 mol) and 2,5-dimethoxytetrahydrofuran (0.07 mol) in acetic acid (100 mL) was refluxed for 1 h with vigorous stirring. After cooling, the reaction mixture was poured into water (300 mL). The precipitate was filtered, washed with water, dissolved in diethyl ether (150 mL), dried over magnesium sulfate, and evaporated to dryness under reduced pressure to give orange crystals, which were recrystallized from petroleum ether. Orange crystals (79%), mp 73 °C. ^{1}H NMR ($CDCl_3$) δ: 7.81 (d, 1H, J = 8.45 Hz, H-3), 7.46 (d, 1H, J = 2.10 Hz, H-6), 7.41 (dd, 1H, J = 8.45 and 2.10 Hz, H-4), 6.77 (dd, 2H, J = 2.15 and 2.15 Hz, H-α), 6.37 (dd, 2H, J = 2.15 and 2.15 Hz, H-β). ^{13}C NMR (CDCl3) δ: 143.0 (C-2), 139.1 (C-5), 135.1 (C-1), 127.5 (C-4), 127.4 (C-α), 126.2 (C-6), 121.0 (C-3), 111.4 (C-β). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

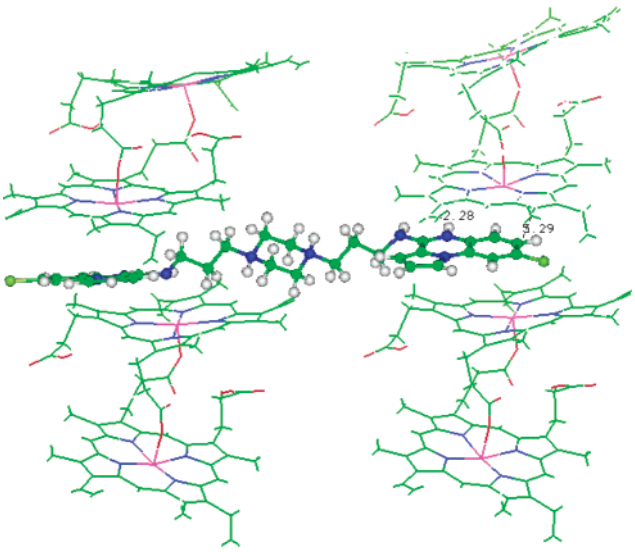


Figure 6. Proposed interaction between compound **4b** and the tetramer. The following distances are between boldfaced atoms: N**H**−H**C**═C(vinyl), 2.28 Å; H$_3$**C**−**C**Cl, 5.29 Å.

1-(4-Methoxy-2-nitrophenyl)pyrrole (8c) was synthesized from **7c** according to the same conditions as those described to obtain **8b**. This gave **8c** as orange crystals (75%), mp 57 °C. ^{1}H NMR ($CDCl_3$) δ: 7.38 (d, 1H, J = 8.85 Hz, H-6), 7.35 (d, 1H, J = 2.75 Hz, H-3), 7.16 (dd, 1H, J = 8.85 and 2.75 Hz, H-5), 6.75 (dd, 2H, J = 2.00 and 2.00 Hz, H-α), 6.32 (dd, 2H, J = 2.00 and 2.00 Hz, H-β), 3.88 (s, 3H, CH_3). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

1-(5-Methoxy-2-nitrophenyl)pyrrole (8d) was synthesized from **7d** according to the same conditions as those described to obtain **8b**. This gave **8d** as orange crystals (78%), mp 48 °C. ^{1}H NMR ($CDCl_3$) δ: 7.94 (d, 1H, J = 8.85 Hz, H-3), 6.91 (dd, 1H, J = 8.85 and 2.45 Hz, H-4), 6.89 (d, 1H, J = 2.45 Hz, H-6), 6.77 (dd, 2H, J = 2.15 and 2.15 Hz, H-α), 6.34 (dd, 2H, J = 2.15 and 2.15 Hz, H-β), 3.89 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 163.9 (C-5), 138.1 (C-1), 137.5 (C-3), 128.2 (C-2), 121.9 (C-α), 113.7 (C-4), 113.4 (C-6), 111.4 (C-β), 56.8 (CH_3O). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

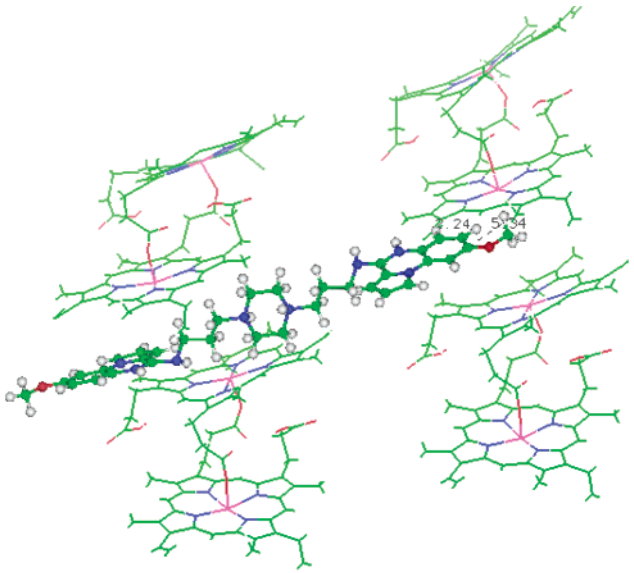


Figure 7. Proposed interaction between compound **4d** and the tetramer. The following distances are between boldfaced atoms: N**H**−H**C**=C(vinyl), 2.24 Å; H_3**C**−**C**OCH_3, 5.34 Å.

1-(2-Amino-5-chlorophenyl)pyrrole (9b). To a solution of 1-(2-nitrophenyl)pyrrole **8b** (0.02 mol) in ethanol (130 mL) was added $BiCl_3$ (0.03 mol). Sodium borohydride (0.16 mol) was added portionwise at 0 °C to the reaction mixture, which was then stirred at room temperature for 2 h. The reaction mixture was then poured into an aqueous hydrochloric acid solution (1 N, 130 mL) and stirred for 1 h. Ethanol was evaporated under reduced pressure. The residue was made alkaline with concentrated aqueous ammonium hydroxide solution and then extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was then recrystallized from petroleum ether to give **9b** as pale-yellow crystals (63%), mp 87 °C. IR (KBr) 3375, 3300 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.14 (d, 1H, J = 2.30 Hz, H-6), 7.12 (dd, 1H, J = 8.05 and 2.30 Hz, H-4), 6.81 (dd, 2H, J = 1.95 and 1.95 Hz, H-a), 6.70 (d, 1H, J = 8.05 Hz, H-3), 6.35 (dd, 2H, J = 1.95 and 1.95 Hz, H-b), 3.72 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ: 140.6 (C-2), 128.3 (C-4), 127.9 (C-1), 126.9 (C-6), 122.4 (C-5), 121.4 (C-α), 116.8 (C-3), 109.8 (C-*β*). Anal. ($C_{10}H_9ClN_2$) C, H, N.

1-(2-Amino-4-methoxyphenyl)pyrrole (9c) was synthesized from **8c** according to the same conditions as those described to obtain **9b**. This gave **9c** as yellow oil (62%). IR (KBr) 3460, 3375 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.06 (d, 1H, J = 8.20 Hz, H-6), 6.78 (dd, 2H, J = 2.05 and 2.05 Hz, H-α), 6.34 (m, 4H, H-3, H-5 and H-*β*), 3.79 (s, 3H, CH_3O), 3.69 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ: 159.9 (C-4), 143.4 (C-2), 128.1 (C-1), 122.1 (C-α), 121.2 (C-6), 109.2 (C-*β*), 103.6 (C-5), 101.1 (C-3), 55.4 (CH_3O). Anal. ($C_{11}H_{12}N_2O$) C, H, N.

1-(2-Amino-5-methoxyphenyl)pyrrole (9d) was synthesized from **7d** according to the same conditions as those described to obtain **9b**. This gave **9d** as pale-yellow crystals (47%), mp 43 °C. IR (KBr) 3435, 3355 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 6.85 (m, 2H, H-3 and H-6), 6.76 (m, 3H, H-6 and H-α), 6.34 (dd, 2H, J = 2.10 and 2.10 Hz, H-*β*), 3.74 (s, 3H, CH_3O), 3.45 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ: 153.1 (C-5), 136.0 (C-1), 128.8 (C-2), 122.2 (C-α), 117.9 (C-3), 115.2 (C-4), 112.8 (C-6), 110.2 (C-*β*), 56.4 (CH_3O). Anal. ($C_{11}H_{12}N_2O$) C, H, N.

8-Chloro-5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one (10b). To a solution of compound **9b** (0.04 mol) in toluene (90 mL) was added triphosgene (0.0134 mol). The reaction mixture was refluxed for 4 h, and nitrogen was bubbled in to drive off the excess of phosgene. The solution was then set aside for 30 min. The heavy crystalline precipitate was filtered off and washed with diethyl ether to give **10b** as white crystals (90%), mp 270 °C. IR (KBr) 3300, 2700 (NH), 1690 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 11.33 (s, 1H, NH), 8.19 (m, 2H, H-1 and H-9), 7.25 (m, 2H, H-6 and H-7), 7.02 (dd, 1H, J = 3.95 and 1.30 Hz, H-3), 6.65 (dd, 1H, J = 3.95 and 2.60 Hz, H-2). ^{13}C NMR (DMSO-d_6) δ: 158.2 (C-4), 131.0 (C-5a), 129.9 (C-3a), 128.8 (C-8), 127.0 (C-9a), 126.7 (C-1), 122.1 (C-7), 121.3 (C-3), 118.4 (C-9), 116.6 (C-6), 115.3 (C-2). Anal. ($C_{11}H_7ClN_2O$) C, H, N.

7-Methoxy-5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one (10c) was synthesized from **9c** according to the same conditions as those described to obtain **10b**. This gave **10c** as orange crystals (84%), mp 252 °C. IR (KBr) 3200, 2700 (NH), 1685 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 11.15 (s, 1H, NH), 8.04 (dd, 1H, J = 2.45 and 0.80 Hz, H-1), 7.90 (d, 1H, J = 8.85 Hz, H-9), 6.97 (dd, 1H, J = 3.60 and 0.80 Hz, H-3), 6.82 (d, 1H, J = 2.60 Hz, H-6), 6.75 (dd, 1H, J = 8.85 and 2.60 Hz, H-8), 6.60 (dd, 1H, J = 3.60 and 2.45 Hz, H-2), 3.75 (s, 3H, CH_3O). ^{13}C NMR (DMSO-d_6) δ: 157.0 (C-4), 155.2 (C-7), 129.7 (C-9a), 122.6 (C-5a), 117.7 (C-1), 116.8 (C-3a), 116.0 (C-6), 112.3 (C-3), 111.0 (C-2), 108.9 (C-9), 100.8 (C-8), 55.4 (CH_3O). Anal. ($C_{12}H_{10}N_2O_2$) C, H, N.

8-Methoxy-5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one (10d) was synthesized from **9d** according to the same conditions as those described to obtain **10b**. This gave **10d** as pale-orange crystals (53%), mp 265 °C. IR (KBr) 3200, 2800 (NH), 1655 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 11.07 (s, 1H, NH), 8.20 (dd, 1H, J = 2.50 and 1.10 Hz, H-1), 7.61 (d, 1H, J = 2.50 Hz, H-9), 7.21 (d, 1H, J = 8.85 Hz, H-6), 6.98 (dd, 1H, J = 3.65 and 1.10 Hz, H-3), 6.89 (dd, 1H, J = 8.85 and 2.50 Hz, H-7), 6.66 (dd, 1H, J = 3.65 and 2.50 Hz, H-2), 3.82 (s, 3H, CH_3O). ^{13}C NMR (DMSO-d_6) δ: 155.3 (C-4), 154.8 (C-8), 123.5 (C-9a), 123.3 (C-5a), 122.3 (C-1), 118.4 (C-3a), 117.6 (C-6), 112.8 (C-3), 112.6 (C-2), 111.4 (C-9), 100.2 (C-7), 56.0 (CH_3O). Anal. ($C_{10}H_9ClN_2$) C, H, N.

4,8-Dichloropyrrolo[1,2-*a*]quinoxaline (11b). A solution of 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one 10b (0.03 mol) in $POCl_3$ (60 mL) was refluxed for 4 h. After removal of excess reactive material under vacuum, the residue was carefully dissolved in water at 0 °C and the resulting solution was made basic with 30% aqueous ammonium hydroxide solution. The precipitate was filtered, dried, and recrystallized from ethyl acetate to give **11b** as white crystals (81%), mp 187 °C. ^1H NMR (DMSO-d_6) δ: 8.57 (m, 1H, H-1), 8.42 (d, 1H, J = 2.15 Hz, H-9), 7.74 (d, 1H, J = 8.65 Hz, H-6), 7.45 (dd, 1H, J = 8.65 and 2.15 Hz, H-7), 7.00 (m, 1H, H-3), 6.95 (m, 1H, H-2). Anal. ($C_{11}H_6Cl_2N_2$) C, H, N.

4-Chloro-7-methoxypyrrolo[1,2-*a*]quinoxaline (11c) was synthesized from **10c** according to the same conditions as those described to obtain **11b**. This gave **11c** as white crystals (92%), mp 133 °C. ^1H NMR (DMSO-d_6) δ: 8.38 (m, 1H, H-1), 8.07 (d, 1H, J = 8.90 Hz, H-9), 7.21 (d, 1H, J = 2.10 Hz, H-6), 7.11 (dd, 1H, J = 8.90 and 2.10 Hz, H-8), 6.89 (m, 1H, H-3), 6.85 (m, 1H, H-2), 3.81 (s, 3H, CH_3O). ^{13}C NMR (DMSO-d_6) δ: 157.0 (C-4), 143.9 (C-7), 135.2 (C-9a), 122.5 (C-5a), 120.9 (C-9), 118.0 (C-3a), 116.7 (C-6), 115.8 (C-1), 114.0 (C-3), 110.5 (C-8), 107.9 (C-2), 55.5 (CH_3O). Anal. ($C_{12}H_9ClN_2O$) C, H, N.

4-Chloro-8-methoxypyrrolo[1,2-*a*]quinoxaline (11d) was synthesized from **10d** according to the same conditions as those described to obtain **11b**. This gave **11d** as beige crystals (83%), mp 126 °C. ^1H NMR (DMSO-d_6) δ: 8.57 (m, 1H, H-1), 7.77 (d, 1H, J = 2.60 Hz, H-9), 7.72 (d, 1H, J = 8.65 Hz, H-6), 7.08 (dd, 1H, J = 8.65 and 2.60 Hz, H-7), 6.98 (m, 2H, H-2 and H-3), 3.93 (s, 3H, CH_3O). ^{13}C NMR (DMSO-d_6) δ: 159.5 (C-4), 140.9 (C-8), 129.9 (C-9a), 128.2 (C-5a), 127.8 (C-3a), 122.8 (C-6), 118.3 (C-1), 114.4 (C-3), 114.0 (C-2), 107.8 (C-9), 98.5 (C-7), 56.1 (CH_3O). Anal. ($C_{12}H_9ClN_2O$) C, H, N.

1,4-Dichloro-7-methoxypyrrolo[1,2-*a*]quinoxaline (12c). A mixture of lactam **10c** (0.006 mol) and phosphorus pentachloride (0.0111 mol) in phosphorus oxychloride (8 mL) was heated under reflux for 2.5 h. The reaction mixture was then evaporated to dryness, the residue was dissolved in water at 0 °C, and the resulting solution was made alkaline with 30% aqueous ammonium hydroxyde solution. The mixture was extracted with methylene chloride. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give yellow crystals (77%), mp 126 °C. ^1H NMR (DMSO-d_6) δ: 8.72 (d, 2H, J = 9.35 Hz, H-9), 7.20 (d, 2H, J = 2.95 Hz, H-6), 6.95

(dd, 2H, J = 9.35 and 2.95 Hz, H-8), 6.90 (d, 2H, J = 4.35 Hz, H-3), 6.62 (d, 2H, J = 4.35 Hz, H-2), 3.83 (s, 3H, CH_3O). ^{13}C NMR (DMSO-d_6) δ: 157.2 (C-4), 147.7 (C-7), 137.0 (C-9a), 123.3 (C-5a), 122.1 (C-9), 116.3 (C-3a), 115.9 (C-6), 114.5 (C-1), 110.8 (C-3), 107.9 (C-8), 107.0 (C-2), 55.5 (CH_3O). Anal. ($C_{12}H_8Cl_2N_2O$) C, H, N.

***N*-(Pyrrolo[1,2-*a*]quinoxalin-4-yl)-*N*′,*N*′-diethyl-1-methylbutane-1,4-diamine (1a).** To **11a** (0.013 mol) was added 5 mL of 5-diethylamino-2-pentylamine. The reaction mixture was heated at 150−160 °C for 4 h and, after cooling, was poured into water (100 mL). The mixture was extracted with diethyl ether. The organic layer was washed with water (150 mL), dried over Na_2SO_4, and evaporated to dryness to give **1a** as an orange oil (58%). IR (KBr) 3215 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.74 (dd, 1H, J = 2.70 and 1.15 Hz, H-1), 7.65 (dd, 1H, J = 7.85 and 1.35 Hz, H-9), 7.61 (dd, 1H, J = 7.85 and 1.35 Hz, H-6), 7.23 (m, 2H, H-7 and H-8), 6.67 (dd, 1H, J = 3.85 and 2.70 Hz, H-2), 6.59 (dd, 1H, J = 3.85 and 1.15 Hz, H-3), 4.91 (d, 1H, J = 8.15 Hz, NH), 4.52 (m, 1H, CH), 2.60 (t, 2H, J = 7.25 Hz, CH_2), 2.48 (q, 4H, J = 7.30 Hz, CH_2), 1.51 (m, 4H, CH_2), 1.32 (d, 3H, J = 6.55 Hz, CH_3), 1.10 (t, 6H, J = 7.30 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 148.7 (C-4), 137.3 (C-5a), 126.8 (C-6), 125.2 (C-7), 125.0 (C-9a), 122.5 (C-8), 119.6 (C-3a), 114.1 (C-9), 113.2 (C-1), 112.1 (C-3), 101.9 (C-2), 52.8 (CH), 46.8 (CH_2), 45.7 (CH_2), 35.2 (CH_2), 23.7 (CH_3), 21.1 (CH_2), 11.5 (CH_3). Anal. (**1a**·2 oxalate ($C_{20}H_{28}N_4$·$2C_2H_2O_4$)) C, H, N.

***N*-(8-Chloropyrrolo[1,2-*a*]quinoxalin-4-yl)-*N*′,*N*′-diethyl-1-methylbutane-1,4-diamine (1b)** was synthesized from **11b** according to the same conditions as those described to obtain **1a**. This gave **1b** as an orange oil (79%). IR (KBr) 3435 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.64 (dd, 1H, J = 2.90 and 1.25 Hz, H-1), 7.62 (d, 1H, J = 2.30 Hz, H-9), 7.50 (d, 1H, J = 8.70 Hz, H-6), 7.19 (dd, 1H, J = 8.70 and 2.30 Hz, H-7), 6.67 (dd, 1H, J = 3.85 and 2.90 Hz, H-2), 6.58 (dd, 1H, J = 3.85 and 1.25 Hz, H-3), 4.99 (d, 1H, J = 8.10 Hz, NH), 4.48 (m, 1H, CH), 2.50 (t, 2H, J = 7.20 Hz, CH_2), 2.44 (q, 4H, J = 7.30 Hz, CH_2), 1.57 (m, 4H, CH_2), 1.28 (d, 3H, J = 6.50 Hz, CH_3), 0.97 (t, 6H, J = 7.30 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 148.7 (C-4), 136.0 (C-5a), 127.7 (C-6), 127.2 (C-7), 125.7 (C-9a), 125.2 (C-8), 119.4 (C-3a), 114.4 (C-9), 113.4 (C-1), 112.7 (C-3), 102.5 (C-2), 52.8 (CH), 46.8 (CH_2), 45.7 (CH_2), 35.2 (CH_2), 23.7 (CH_3), 21.2 (CH_2), 11.5 (CH_3). Anal. (**1b**·2 oxalate ($C_{20}H_{27}ClN_4$·$2C_2H_2O_4$)) C, H, N.

***N*-(7-Methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-*N*′,*N*′-diethyl-1-methylbutane-1,4-diamine (1c)** was synthesized from **11c** according to the same conditions as those described to obtain **1a**. This gave **1c** as an orange oil (65%). IR (KBr) 3340 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.64 (dd, 1H, J = 2.60 and 1.30 Hz, H-1), 7.53 (d, 1H, J = 8.85 Hz, H-9), 7.08 (d, 1H, J = 2.85 Hz, H-6), 6.75 (dd, 1H, J = 8.85 and 2.85 Hz, H-8), 6.68 (dd, 1H, J = 3.90 and 1.30 Hz, H-3), 6.61 (dd, 1H, J = 3.90 and 2.60 Hz, H-2), 5.15 (d, 1H, J = 7.95 Hz, NH), 4.51 (m, 1H, CH), 3.84 (s, 3H, CH_3O), 2.63 (t, 2H, J = 7.20 Hz, CH_2), 2.52 (q, 4H, J = 7.30 Hz, CH_2), 1.66 (m, 4H, CH_2), 1.34 (d, 3H, J = 6.60 Hz, CH_3), 1.14 (t, 6H, J = 7.30 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 157.9 (C-4), 149.9 (C-7), 139.0 (C-9a), 120.3 (C-5a), 119.8 (C-9), 114.7 (C-3a), 114.5 (C-6), 112.5 (C-1), 111.5 (C-2), 109.6 (C-8), 102.8 (C-2), 56.2 (CH_3O), 53.2 (CH), 47.4 (CH_2), 46.1 (CH_2), 35.4 (CH_2), 23.5 (CH_3), 22.0 (CH_2), 11.3 (CH_3). Anal. (**1c**·2 oxalate ($C_{21}H_{30}N_4O$·$2C_2H_2O_4$)) C, H, N.

Bis{*N*-(pyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}methylamine (2a). To a solution of **11a** (0.013 mol) in dimethylformamide (35 mL) were added K_2CO_3 (0.0143 mol) and then 3,3′-diamino-*N*-methyldipropylamine (0.0065 mol). The reaction mixture was heated at 120−130 °C for 4 h and, after cooling, was poured into water (100 mL). The precipitate was filtered, washed with water, and dissolved in dichloromethane. The organic layer was washed with water (150 mL), dried over Na_2SO_4, and evaporated to dryness to give **2a** as yellow crystals (64%), mp 48 °C. IR (KBr) 3255 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.71 (m, 2H, H-1), 7.66 (d, 2H, J = 7.75 Hz, H-9), 7.61 (d, 2H, J = 7.75 Hz, H-6), 7.26 (t, 2H, J = 7.75 Hz, H-8), 7.17 (t, 2H, J = 7.75 Hz, H-7), 6.62 (m, 2H, H-3), 6.56 (m, 2H, H-2), 6.28 (t, 2H, J = 4.90 Hz, NH), 3.75 (m, 4H, CH_2), 2.59 (t, 4H, J = 6.45 Hz, CH_2), 2.36 (s, 3H, CH_3), 1.93 (qt, 4H, J = 6.45 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 149.5 (C-4), 137.2 (C-5a), 126.6 (C-6), 125.2 (C-7), 125.1 (C-9a), 122.5 (C-8), 119.7 (C-3a), 114.1 (C-9), 113.3 (C-1), 112.2 (C-3), 102.3 (C-2), 56.9 (CH_2), 42.2 (CH_2), 40.4 (CH_2), 26.3 (CH_2). Anal. (**2a**·3 oxalate ($C_{29}H_{31}N_7$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-chloropyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}methylamine (2b) was synthesized from **11b** according to the same conditions as those described to obtain **2a**. This gave **2b** as yellow crystals (72%), mp 107 °C. IR (KBr) 3435 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.56 (m, 4H, H-1 and H-9), 7.46 (d, 2H, J = 8.55 Hz, H-6), 7.15 (dd, 2H, J = 8.55 and 2.15 Hz, H-7), 6.55 (m, 4H, H-2 and H-3), 6.37 (t, 2H, J = 4.70 Hz, NH), 3.69 (m, 4H, CH_2), 2.59 (t, 4H, J = 6.30 Hz, CH_2), 2.33 (s, 3H, CH_3), 1.88 (qt, 4H, J = 6.30 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 149.4 (C-4), 135.9 (C-5a), 127.5 (C-6), 127.2 (C-7), 125.7 (C-9a), 125.2 (C-8), 119.5 (C-3a), 114.3 (C-9), 113.4 (C-1), 112.7 (C-3), 102.8 (C-2), 56.9 (CH_2), 42.3 (CH_2), 40.5 (CH_2), 26.3 (CH_2). Anal. (**2b**·3 oxalate ($C_{29}H_{29}Cl_2N_7$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(7-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}methylamine (2c) was synthesized from **11c** according to the same conditions as those described to obtain **2a**. This gave **2c** as yellow crystals (70%), mp 63 °C. IR (KBr) 3240 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.61 (dd, 2H, J = 2.60 and 1.35 Hz, H-1), 7.53 (d, 2H, J = 8.85 Hz, H-9), 7.11 (d, 2H, J = 2.75 Hz, H-6), 6.76 (dd, 2H, J = 8.85 and 2.75 Hz, H-8), 6.54 (dd, 2H, J = 3.65 and 1.35 Hz, H-3), 6.52 (dd, 2H, J = 3.65 and 1.35 Hz, H-2), 6.31 (t, 2H, J = 4.55 Hz, NH), 3.81 (s, 6H, CH_3O), 3.74 (m, 4H, CH_2), 2.57 (t, 4H, J = 6.40 Hz, CH_2), 2.34 (s, 3H, CH_3), 1.91 (qt, 4H, J = 6.40 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 157.2 (C-4), 149.9 (C-7), 138.4 (C-9a), 119.6 (C-5a), 119.2 (C-9), 114.1 (C-3a), 113.8 (C-6), 111.9 (C-9), 110.9 (C-3), 108.8 (C-8), 102.0 (C-2), 56.9 (CH_2), 55.5 (CH_3O), 42.2 (CH_3O), 42.2 (CH_3), 40.4 (CH_2), 26.3 (CH_2). Anal. (**2c**·3 oxalate ($C_{31}H_{35}N_7O_2$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}methylamine (2d) was synthesized from **11d** according to the same conditions as those described to obtain **2a**. This gave **2d** as yellow crystals (67%), mp 51 °C. IR (KBr) 3410 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.63 (m, 2H, H-1), 7.54 (d, 2H, J = 8.70 Hz, H-6), 7.24 (d, 2H, J = 2.65 Hz, H-9), 6.86 (dd, 2H, J = 8.70 and 2.65 Hz, H-7), 6.60 (m, 2H, H-3), 6.51 (m, 2H, H-2), 6.01 (t, 2H, J = 4.50 Hz, NH), 3.87 (s, 6H, CH_3O), 3.74 (m, 4H, CH_2), 2.53 (t, 4H, J = 6.60 Hz, CH_2), 2.36 (s, 3H, CH_3), 1.89 (qt, 4H, J = 6.60 Hz, CH_2). Anal. (**2d**·3 oxalate ($C_{31}H_{35}N_7O_2$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-chloropyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}amine (3b). To a solution of **11b** (0.013 mol) in dimethylformamide (35 mL) were added K_2CO_3 (0.0143 mol) and then *N*-(3-aminopropyl)-1,3-propanediamine (0.0065 mol). The reaction mixture was heated at 120−130 °C for 4 h and, after cooling, was poured into water (100 mL). The precipitate was filtered, washed with water, and dissolved in dichloromethane. The organic layer was washed with water (150 mL), dried over Na_2SO_4, and evaporated to dryness to give **3b** as yellow crystals (65%), mp 44 °C. IR (KBr) 3420 and 3315 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.63 (m, 4H, H-1 and H-9), 7.49 (d, 2H, J = 8.60 Hz, H-6), 7.17 (dd, 2H, J = 8.60 and 2.00 Hz, H-7), 6.62 (m, 4H, H-2 and H-3), 6.22 (m, 2H, NH), 3.78 (m, 4H, CH_2), 2.80 (t, 4H, J = 6.10 Hz, CH_2), 1.91 (qt, 4H, J = 6.10 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 149.5 (C-4), 135.8 (C-5a), 127.4 (C-6), 127.2 (C-7), 125.7 (C-9a), 125.2 (C-8), 119.4 (C-3a), 114.4 (C-9), 113.4 (C-1), 112.7 (C-3), 103.0 (C-2), 48.1 (CH_2), 39.8 (CH_2), 29.1 (CH_2). Anal. (**3b**·3 oxalate ($C_{28}H_{27}Cl_2N_7$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(7-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}amine (3c) was synthesized from **11c** according to the same conditions as those described to obtain **3b**. This gave **3c** as yellow crystals (71%), mp 56 °C. IR (KBr) 3395 and 3305 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 7.65 (m, 2H, H-1), 7.55 (d, 2H, J = 8.85 Hz, H-9), 7.12 (d, 2H, J = 2.80 Hz, H-6), 6.77 (dd, 2H, J = 8.85 and 2.80 Hz, H-8), 6.60 (m, 4H, H-2

and H-3), 6.25 (m, 2H, NH), 3.85 (m, 4H, CH_2), 3.82 (s, 6H, CH_3O), 2.78 (t, 4H, J = 6.10 Hz, CH_2), 1.90 (qt, 4H, J = 6.10 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 157.3 (C-4), 149.9 (C-7), 138.4 (C-9a), 119.6 (C-5a), 119.1 (C-9), 114.1 (C-3a), 113.9 (C-6), 111.9 (C-1), 110.9 (C-3), 108.8 (C-8), 102.1 (C-2), 55.5 (CH_3O), 48.2 (CH_2), 39.8 (CH_2), 29.8 (CH_2). Anal. (**3c**·3 oxalate ($C_{30}H_{33}N_7O_2$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}amine (3d) was synthesized from **11d** according to the same conditions as those described to obtain **3b**. This gave **3d** as yellow crystals (65%), mp 49 °C. IR (KBr) 3410 and 3350 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.65 (dd, 2H, J = 2.60 and 1.15 Hz, H-1), 7.53 (d, 2H, J = 8.75 Hz, H-6), 7.15 (d, 2H, J = 2.80 Hz, H-9), 6.87 (dd, 2H, J = 8.75 and 2.80 Hz, H-7), 6.64 (dd, 2H, J = 3.75 and 1.15 Hz, H-3), 6.60 (dd, 2H, J = 3.75 and 2.60 Hz, H-2), 5.94 (m, 2H, NH), 3.87 (s, 6H, CH_3O), 3.76 (m, 4H, CH_2), 2.81 (t, 4H, J = 6.15 Hz, CH_2), 1.93 (qt, 4H, J = 6.15 Hz, CH_2). Anal. (**3d**·3 oxalate ($C_{30}H_{33}N_7O_2$·$3C_2H_2O_4$)) C, H, N.

Bis{*N*-(pyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}-piperazine (4a). To a solution of **11a** (0.013 mol) in dimethylformamide (35 mL) were added K_2CO_3 (0.0143 mol) and then 1,4-bis(3-aminopropyl)piperazine (0.0065 mol). The reaction mixture was heated at 120−130 °C for 4 h and, after cooling, was poured into water (100 mL). The precipitate was filtered, washed with water, and dissolved in dichloromethane. The organic layer was washed with water (150 mL), dried over Na_2SO_4, and evaporated to dryness to give **4a** as pale-yellow crystals (61%), mp 203 °C. IR (KBr) 3230 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.75 (m, 2H, H-1), 7.68 (d, 2H, J = 7.75 Hz, H-9), 7.64 (d, 2H, J = 7.75 Hz, H-6), 7.28 (t, 2H, J = 7.75 Hz, H-8), 7.22 (t, 2H, J = 7.75 Hz, H-7), 6.71 (m, 6H, NH), 3.79 (m, 4H, CH_2), 3.65 (m, 12H, CH_2), 1.90 (qt, 4H, J = 5.85 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 149.6 (C-4), 137.3 (C-5a), 126.6 (C-6), 125.2 (C-7), 125.1 (C-9a), 122.5 (C-8), 120.2 (C-3a), 114.1 (C-9), 113.3 (C-1), 112.1 (C-3), 102.6 (C-2), 58.5 (CH_2), 53.6 (CH_2), 41.4 (CH_2), 24.8 (CH_2). Anal. (**4a**·4 oxalate ($C_{32}H_{36}N_8$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-chloropyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}piperazine (4b) was synthesized from **11b** according to the same conditions as those described to obtain **4a**. This gave **4b** as yellow crystals (84%), mp 226 °C. IR (KBr) 3290 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.68 (m, 2H, H-1), 7.65 (d, 2H, J = 2.20 Hz, H-9), 7.53 (d, 2H, J = 8.60 Hz, H-6), 7.21 (dd, 2H, J = 8.60 and 2.20 Hz, H-7), 6.80 (t, 2H, J = 4.90 Hz, NH), 6.71 (m, 4H, H-2 and H-3), 3.77 (m, 4H, CH_2), 2.66 (m, 12H, CH_2), 1.91 (qt, 4H, J = 5.90 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 149.5 (C-4), 135.8 (C-5a), 127.6 (C-6), 127.2 (C-7), 125.7 (C-9a), 125.5 (C-8), 119.6 (C-3a), 114.3 (C-9), 113.4 (C-1), 112.6 (C-3), 103.0 (C-2), 58.5 (CH_2), 53.6 (CH_2), 41.4 (CH_2), 24.7 (CH_2). Anal. (**4b**·4 oxalate ($C_{32}H_{34}Cl_2N_8$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(7-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}piperazine (4c) was synthesized from **11c** according to the same conditions as those described to obtain **4a**. This gave **4c** as yellow crystals (63%), mp 199 °C. IR (KBr) 3255 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.68 (m, 2H, H-1), 7.58 (d, 2H, J = 8.85 Hz, H-9), 7.14 (d, 2H, J = 2.80 Hz, H-6), 6.79 (m, 4H, H-8 and H-3), 6.68 (m, 4H, *H-2* and NH), 3.86 (s, 6H, CH_3O), 3.79 (m, 4H, CH_2), 2.66 (m, 12H, CH_2), 1.91 (qt, 4H, J = 5.95 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 157.3 (C-4), 150.0 (C-7), 138.5 (C-9a), 119.6 (C-5a), 119.3 (C-9), 114.1 (C-3a), 113.8 (C-6), 111.7 (C-1), 110.9 (C-3), 108.8 (C-8), 102.2 (C-2), 58.6 (CH_2), 55.6 (CH_3O), 53.6 (CH_2), 41.5 (CH_2), 24.7 (CH_2). Anal. (**4c**·4 oxalate ($C_{34}H_{40}N_8O_2$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(8-methoxypyrrolo[1,2-*a*]quinoxalin-4-yl)-3-aminopropyl}piperazine (4d) was synthesized from **11d** according to the same conditions as those described to obtain **4a**. This gave **4d** as yellow crystals (61%), mp 73 °C. IR (KBr) 3325 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.67 (m, 2H, H-1), 7.56 (d, 2H, J = 8.85 Hz, H-6), 7.16 (d, 2H, J = 2.60 Hz, H-9), 6.90 (dd, 2H, J = 8.85 and 2.60 Hz, H-7), 6.68 (m, 4H, H-*2* and H-3), 6.48 (t, 2H, J = 5.05 Hz, NH), 3.88 (s, 6H, CH_3O), 3.75 (m, 4H, CH_2), 2.62 (m, 12H, CH_2), 1.90 (qt, 4H, J = 6.10 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 156.5 (C-4), 149.7 (C-8), 132.0 (C-9a), 128.2 (C-5a), 126.3 (C-9), 120.7 (C-3a), 114.6 (C-6), 112.9 (C-1), 112.7 (C-3), 103.1 (C-7), 99.0 (C-2), 59.1 (CH_2), 56.4 (CH_3O), 54.3 (CH_2), 42.1 (CH_2), 25.7 (CH_2). Anal. (**4d**·4 oxalate ($C_{34}H_{40}N_8O_2$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazin-6-yl)-3-aminopropyl}piperazine (4e) was synthesized from **11e** according to the same conditions as those described to obtain **4a**. This gave **4e** as pale-yellow crystals (34%), mp 215 °C. IR (KBr) 3240 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.20 (m, 4H, H-2 and H-9), 7.87 (dd, 2H, J = 7.95 and 1.50 Hz, H-4), 7.25 (dd, 2H, J = 7.95 and 4.70 Hz, H-3), 6.91 (t, 2H, J = 4.20 Hz, NH), 6.75 (dd, 2H, J = 3.75 and 1.45 Hz, H-7), 6.71 (m, 2H, H-8), 3.80 (m, 4H, CH_2), 2.68 (m, 12H, CH_2), 1.92 (qt, 4H, J = 5.90 Hz, CH_2). Anal. (**4e**·4 oxalate ($C_{30}H_{34}N_{10}$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(2-methoxypyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazin-6-yl)-3-aminopropyl}piperazine (4f) was synthesized from **11f** according to the same conditions as those described to obtain **4a**. This gave **4f** as yellow crystals (21%), mp 201 °C. IR (KBr) 3230 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.10 (m, 2H, H-9), 7.83 (d, 2H, J = 8.50 Hz, H-4), 6.73 (d, 2H, J = 8.50 Hz, H-3), 6.71 (m, 4H, H-7 and H-8), 6.59 (t, 2H, J = 5.85 Hz, NH), 4.01 (s, 6H, OCH_3), 3.73 (m, 4H, CH_2), 2.63 (m, 12H, CH_2), 1.92 (qt, 4H, J = 5.85 Hz, CH_2). Anal. (**4f**·4 oxalate ($C_{32}H_{38}N_{10}O_2$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(1-chloro-7-methoxypyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}piperazine (5) was synthesized from **12c** according to the same conditions as those described to obtain **4a**. This gave **5** as yellow crystals (60%), mp 128 °C. IR (KBr) 3230 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.77 (d, 2H, J = 9.30 Hz, H-9), 7.12 (d, 2H, J = 2.85 Hz, H-6), 6.76 (dd, 2H, J = 9.30 and 2.85 Hz, H-8), 6.70 (m, 2H, NH), 6.65 (d, 2H, J = 4.20 Hz, H-3), 6.54 (d, 2H, J = 4.20 Hz, H-2), 3.86 (s, 6H, CH_3O), 3.75 (m, 4H, CH_2), 2.64 (m, 12H, CH_2), 1.89 (qt, 4H, J = 5.80 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 157.8 (C-4), 150.0 (C-7), 138.4 (C-9a), 121.1 (C-5a), 120.1 (C-9), 116.8 (C-3a), 115.0 (C-6), 112.9 (C-1), 110.7 (C-3), 109.5 (C-8), 102.5 (C-2), 59.2 (CH_2), 56.1 (CH_3O), 54.2 (4CH_2), 42.1 (CH_2), 25.3 (CH_2). Anal. (**5c**·4 oxalate ($C_{34}H_{38}Cl_2N_8O_2$·$4C_2H_2O_4$)) C, H, N.

Bis{*N*-(pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazin-5-yl)-3-aminopropyl}piperazine (6) was synthesized from **13** according to the same conditions as those described to obtain **4a**. This gave **6** as orange crystals (52%), mp 197 °C. IR (KBr) 3320 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.31 (m, 2H, H-8), 7.20 (d, 2H, J = 5.60 Hz, H-2), 6.95 (d, 2H, J = 5.60 Hz, H-3), 6.70 (m, 4H, H-6 and H-7), 6.52 (t, 2H, J = 5.80 Hz, NH), 3.71 (m, 4H, CH_2), 2.64 (m, 12H, CH_2), 1.90 (qt, 4H, J = 6.00 Hz, CH_2). Anal. (**6**·4 oxalate ($C_{28}H_{32}N_8S_2$·$4C_2H_2O_4$)) C, H, N.

X-ray Data. The structures of compounds **4b** and **4e** have been established by X-ray crystallography (Figure 1).[48] Colorless single crystals (0.50 × 0.15 × 0.10 mm^3) of **4b** were obtained by slow evaporation from a methanol/chloroform (40/60) solution: triclinic, space group $P\bar{1}$, a = 9.597(3) Å, b = 10.516(2) Å, c = 10.846(1) Å, α = 67.37(1)°, β = 72.68(2)°, γ = 83.73(2)°, V = 964.5(4) Å^3, Z = 1, ρ(calcd) = 1.447 Mg·m^{-3}, FW = 840.32 for $C_{32}H_{34}Cl_2N_8$·$2CHCl_3$, F(000) = 432. Colorless single crystals (0.37 × 0.15 × 0.01 mm^3) of **4e** were obtained by slow evaporation from a methanol/chloroform (30/70) solution: monoclinic, space group $P21/c$, a = 12.078(1) Å, b = 5.617(1) Å, c = 19.647(1) Å, α = 90.0°, β = 93.65(2)°, γ = 90.0°, V = 1330.2(3) Å^3, Z = 2, ρ(calcd) = 1.335 Mg·m^{-3}, FW = 534.68 for $C_{30}H_{34}N_{10}$, F(000) = 568. The unit cell dimensions were determined using the least-squares fit from 25 reflections (25° < θ < 35°). Intensities were collected with an Enraf-Nonius CAD-4 diffactometer using the Cu Kα radiation and a graphite monochromator up to θ = 55°. No intensity variation of two standard reflections monitored every 90 min was observed. The data were corrected for Lorentz and polarization effects and for empirical absorption correction.[54] The structure was solved by direct methods Shelx 86[55] and refined using the Shelx 93[56] suite of programs.

Partition Coefficients: log *D* (pH 7.4 or pH 5.0). The relative log D at pH 7.4 and 5.0 of each compound in this study was assessed by the micro-HPLC method.[45] These determinations were performed with a chromatographic apparatus

(Waters, Milford, MA) equipped with a model 501 constant flow pump, a model 2487 ultraviolet detector ($\lambda = 254$ nm), and a 746 data module integrator. A reversed-phase column was used: a Waters Spherisorb S5C8 (4.6 mm × 150 mm, 5 μm particle size) with a mobile phase consisting of acetonitrile−acetate buffer (pH 4) (80:20, v/v). The column was maintained at 60 °C.

The compounds were partitioned between 1-octanol (HPLC grade) and phosphate buffer (pH 7.4) or acetate buffer (pH 5.0). Octanol was presaturated with buffer, and conversely, the buffer was presaturated with octanol. An amount of 1 mg of each compound was dissolved in an adequate volume of methanol in order to achieve 1 mg/mL stock solutions. Then an appropriate aliquot of these methanolic solutions was dissolved in buffer to obtain final concentrations ranging from 50 to 250 μg/mL. Under the above-described chromatographic conditions, 20 μL of this aqueous phase was injected into the chromatograph, leading to the determination of a peak area before partitioning (W_0).

In scew-capped tubes, 1 mL of the aqueous phase (V_{aq}) was then added to 5μL of *n*-octanol (V_{oct}). The mixture was shaken by mechanical rotation during 30 min. Then the centrifugation was achieved at 3000 rpm in 10 min. An amount of 20 μL of the lower phase was injected into the chromatograph column. This led to the determination of a peak area after partitioning (W_1). The log *D* value was determined by the formula

$$\log D = \log\left[\frac{(W_0 - W_1)V_{aq}}{W_1 V_{oct}}\right]$$

Molecular Modeling. All molecular modeling was carried out on a Indigo 2 R44OO silicon graphics workstation. In our studies, we have employed the method of simulated annealing. Molecular dynamics (MD) runs at 300 K for a simulation time of 5 ps (time step of 1 fs) were performed for each drug molecule, and the resulting structure was energy-minimized to gain a low-energy conformation.[57,58]

This process was repeated until 10 structures per molecule were generated. The rmsd values of the three final selected conformations are close together, less than 1.129 638 Å (2.150 644 Å) for **4b** (**4d**) and less than 2.859 583 Å for hematin molecular unit model. For each MD calculation, the last minimized structure in the set was used as the next starting point. Each of the structures was finally minimized at the restricted Hartee-Fock (RHF) level using the PM3 basis set procedure implemented in GAMESS-US.[59]

Biological Assays. In Vitro *P. falciparum* Culture and Drug Assays. *P. falciparum* strains were maintained continuously in culture on human erythrocytes as described by Trager and Jensen.[60] In vitro antiplasmodial activity was determined using a modification of the semiautomated microdilution technique of Desjardins et al.[61] *P. falciparum* CQ-sensitive (Thai/Thailand) and CQ-resistant (FcB1R/Colombia and K1/Thailand) strains were used in sensitivity testing. Stock solutions of CQ diphosphate and test compounds were prepared in sterile distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and added to asynchronous parasite cultures (1% parasitemia and 1% final hematocrite) in 96-well plates for 24 h, at 37 °C, prior to the addition of 0.5 μCi of [^{3}H]hypoxanthine (1−5 Ci/mmol; Amersham, Les Ulis, France) per well, for 24 h. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture (without drug) maintained on the same plate. The concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration−response curve, and the results were expressed as the mean ± the standard deviations determined from several independent experiments. The DMSO concentration never exceeded 0.1% and did not inhibit the parasite growth.

β-Hematin Formation Assay. Method A. The drug effects upon β-hematin formation were assessed according to Raynes et al.[41] The haemozoin content was determined using the procedure of Chou and Fitch.[62] Briefly, a 50 μL aliquot of insoluble trophozoite material of the FcB1R strain (approximately equivalent to 4×10^7 parasites) was added to 900 μL of a haem−acetate mixture (0.3 mM bovine hematin, 60 mM sodium acetate, pH 5). Samples of 50 μL of drug solution at different concentrations were mixed with the other components. Samples without drug constituted controls. All of the samples contained the same amount of DMSO (1%). Following incubation for 4 h, at 37 °C, the samples were centrifuged at 27000*g*, 15 min, at 4 °C. The pellet was resuspended in 1 mL of buffer A (68 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 5 mM glucose, 50 mM sodium phosphate, pH 7.4) and repelleted. The pellet was introduced to 2.5% SDS in buffer A and sonicated for 10 min. The polymerized haem was collected by centrifugation at 27000*g*, 30 min, at 20 °C. The pellet was then washed four times before being resuspended in 900 μL of 2.5% SDS in buffer A and 100 μL of 1 M NaOH was added to dissolve the polymerized haem. Following incubation for 1 h, the concentration of haemozoin was determined by measuring the absorbance at 404 nm.[63] The amount of haemozoin formed during the incubation period was corrected for the endogenous haemozoin of the trophozoite preparation, and the percentage of inhibition of β-hematin formation was determined by comparison with samples maintained without inhibitor. Data presented are the mean of triplicate experiments.

Method B. Experiments were carried out in duplicate in 96 deep wells. In each well, samples of 250 μL of a solution of 700 μM of hemin in 25 mM NaOH were distributed, followed by 250 μL of a suspension of 1 mM 1-monooleoylglycerol in 90 mM sodium acetate with 1% DMSO, at pH 5, extemporaneously prepared. Drugs were added from DMSO stock solutions (5 μL). Microplates were incubated for 24 h at 37 °C. Controls contained an equal amount of DMSO. Following incubation, the samples were centrifuged at 4000 rpm at 4 °C for 30 min. The pellet of β-hematin was washed with 10 mM sodium phosphate, pH 7.4, containing 2.5% SDS and was vortexed for 10 min at 20 °C before repelleting until the supernatant was colorless (five times). Dissolution of β-hematin was achieved by addition of 450 μL of 10 mM sodium phosphate, pH 7.4, containing 2.5% SDS and 25 μL of 1 M NaOH. Concentration of residual haem was calculated from absorbance at 405 nm.

Cytotoxicity Test on Mammalian L6 Cells. Samples of 10^4 L6 cells per well in 96-well plates were maintained in the presence of different concentrations of inhibitors for up to 5 days. Cell proliferation was determined by using the XTT-based colorimetric assay (Cell proliferation kit II, Boehringer Mannheim S.A., Meylan, France). Control cultures received an equivalent amount of DMSO instead of inhibitor. Inhibition of proliferation was determined by comparison to control cultures.

Supporting Information Available: Figures S1 and S2 showing the crystal structures of **4b** and **4e** and Tables S1−S11 listing crystallographic results. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0310840