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Original article

New ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives: Synthesis, and *in vitro* antimalarial activity – Part II

Jean Guillon [a,b,*], Elisabeth Mouray [c], Stéphane Moreau [a,b], Catherine Mullié [d], Isabelle Forfar [a,b], Vanessa Desplat [a,b], Solene Belisle-Fabre [a,b], Noël Pinaud [e], François Ravanello [a,b], Augustin Le-Naour [a,b], Jean-Michel Léger [a,b], Grace Gosmann [f], Christian Jarry [a,b], Gérard Déléris [a,b], Pascal Sonnet [d], Philippe Grellier [c]

[a] *Université Bordeaux Segalen, Pharmacochimie, FRE 3396, F-33000 Bordeaux, France*
[b] *CNRS, Pharmacochimie, FRE 3396, F-33000 Bordeaux, France*
[c] *FRE 3206 CNRS Muséum National d'Histoire Naturelle, Département RDDM, 61 rue Buffon, 75231 Paris Cedex 05, France*
[d] *Laboratoire des Glucides, UMR-CNRS 6219, Faculté de Pharmacie, Université de Picardie Jules Verne, 1 Rue des Louvels, 80037 Amiens Cedex 1, France*
[e] *ISM – CNRS UMR 5255, Université de Bordeaux, 351 cours de la Libération, 33405 Talence cedex, France*
[f] *Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, Porto Alegre 90610-000, RS, Brazil*

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ABSTRACT

Following our search for antimalarial compounds, novel series of ferrocenyl-substituted pyrrolo[1,2-*a*]quinoxalines **1-2** were synthesized from ferrocene-carboxaldehyde and tested for their *in vitro* activity upon the erythrocytic development of *Plasmodium falciparum* strains with different chloroquine-resistance status. The ferrocenic pyrrolo[1,2-*a*]quinoxalines **1-2** were prepared in 6 or 9 steps through a Barton–Zard reaction. Promising pharmacological results against FcB1, K1 and F32 strains were obtained with ferrocenyl pyrrolo[1,2-*a*]quinoxalines **1j-l** linked by a bis-(3-aminopropyl)piperazine linker substituted by a nitrobenzyl moiety.

1. Introduction

Malaria is one of the most prevailing and fatal infectious diseases of the world and has been a public health problem in about 90 countries; whereas, approximately 40% of the world population is under risk of contamination. It causes between 1.2 and 2.7 million deaths each year [1]. Four species of *Plasmodium* cause malaria in human beings; however *Plasmodium falciparum* is the most dangerous of these infections [2–4]. Nevertheless, the increasing prevalence of multiple drug resistant strains of *P. falciparum* in the most malaria endemic areas has significantly reduced the efficacy of current antimalarial drugs for prophylaxis and treatment of this disease. For instance, resistance to the inexpensive antimalarial mainstays, such as chloroquine, is worldwide. Similarly, resistance to mefloquine, which was proposed as the drug of choice for chloroquine-resistant malaria, has been reported from Africa and Southeast Asia [5,6]. Therefore, new antimalarial agents based on novel mode of action are required to overcome the emergence of resistance and to control an ever-increasing number of epidemics caused by the malaria parasite [7]. For several years, it was proposed a strategy for the development of organometallic-based antimalarial drugs [8–11]. Given the avidity of *Plasmodium* for free iron, it was postulated that an effective way of removing the chloroquine resistance of parasites might be the addition of iron to a chloroquine molecule and that a ferrocene moiety will permit to vectorize the drug to the selected target. Hence, some new organometallic compounds including a ferrocene nucleus (dicyclopentadienyl iron) incorporated on chloroquine were designed, which led to the discovery of ferroquine (FQ) and of structural analogues (Fig. 1) [12–16].

Using a strategy similar to the design of FQ, the quinuclidinyl and the piperidinyl side chains of quinine and mefloquine were respectively substituted with a ferrocene moiety while maintaining a basic amino group (Fig. 1) [13,17,18]. This strategy, which is based on the incorporation of a ferrocenyl moiety into the standard drug,

* Corresponding author. Université Bordeaux Segalen, Pharmacochimie, FRE 3396, F-33000 Bordeaux, France. Tel.: +33 5 57 57 46 95; fax: +33 5 57 57 13 52.
E-mail address: jean.guillon@u-bordeaux2.fr (J. Guillon).

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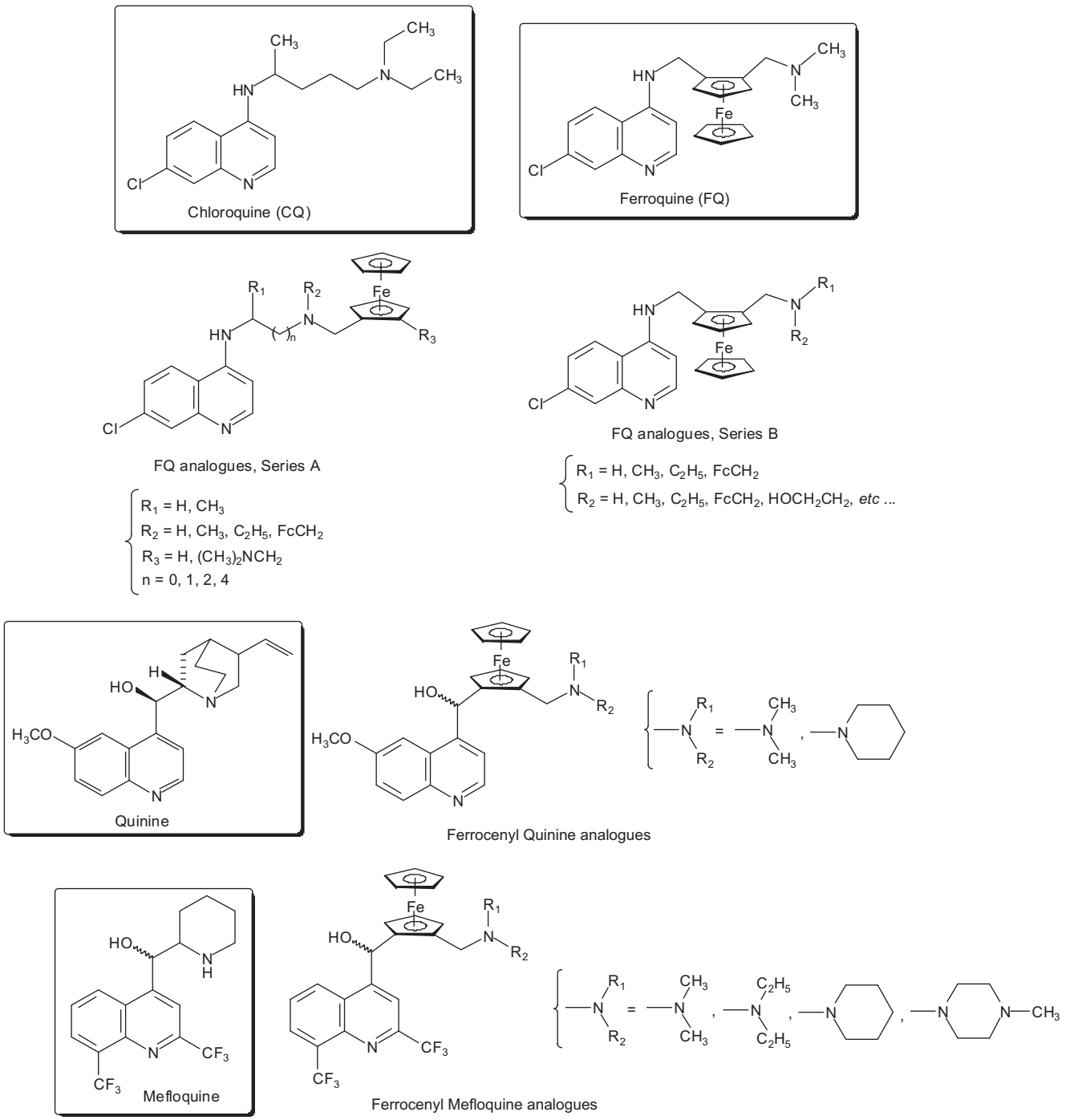


Fig. 1. Structure of chloroquine (CQ), ferroquine (FQ), FQ analogues (Series A-B), quinine, ferrocenyl quinine analogues, mefloquine and ferrocenyl mefloquine analogues.

was also applied to the design and synthesis of new ferrocene-derived artemisinins [13,19], ferrocenyl atovaquone analogues [13,20] or ferrocenyl ciprofloxacin derivatives [21] (Fig. 2).

In the course of our work devoted to discover new compounds employed in the antimalarial chemotherapy, we previously identified a series of substituted ferrocenyl pyrrolo[1,2-*a*]quinoxaline derivatives (Series A) designed as interesting bioactive isosteres of ferrocenyl quinoline derivatives (Fig. 3) [22]. Thus, taking into account our experience in the field of the synthesis of new bioactive heterocyclic compounds based on our pyrrolo[1,2-a] quinoxaline heterocyclic core [22–26], we used the pyrrolo[1,2-*a*] quinoxaline moiety as a template for the design of new derivatives **1** in which the ferrocene nucleus is directly incorporated in position 3 of the heterocyclic core. Moreover, the pyrrolo[1,2-*a*]quinoxaline moiety was linked through a bis-(3-aminopropyl) piperazine *via* a "pseudo-amidinic" bond to a substituted benzyl group containing a hydroxyl function in its ortho position. Hence, previous data showing that several chloroquine analogues containing an intramolecular hydrogen-bonding motif in the basic side chain of the 4-aminoquinoline nucleus were potent against multidrug-resistant *P. falciparum* led to the exploration of the importance of this motif [27,28].

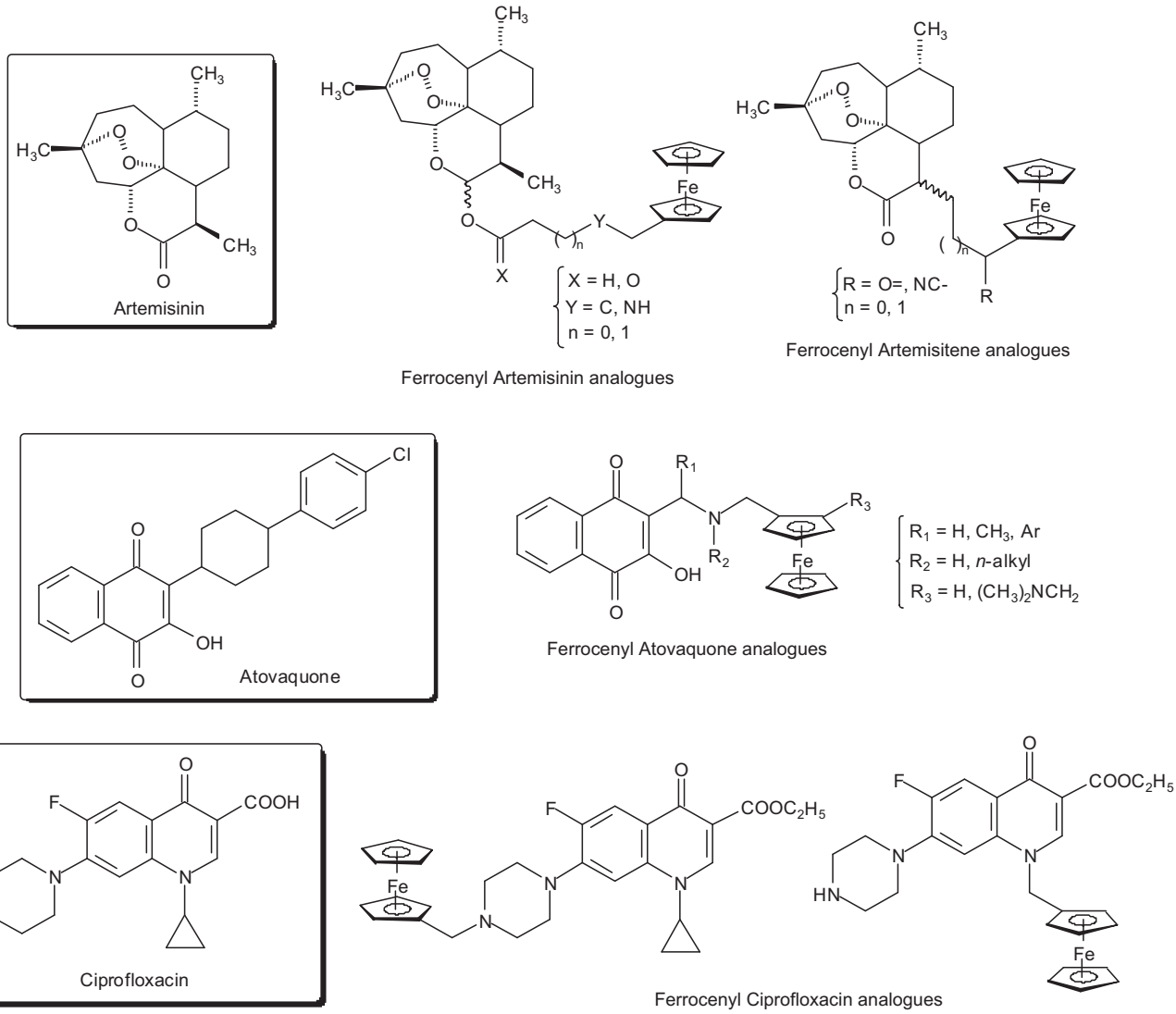


Fig. 2. Structure of artemisinin, ferrocenyl artemisinin analogues, atovaquone, ferrocenyl atovaquone analogues, ciprofloxacin and ferrocenyl ciprofloxacin analogues.

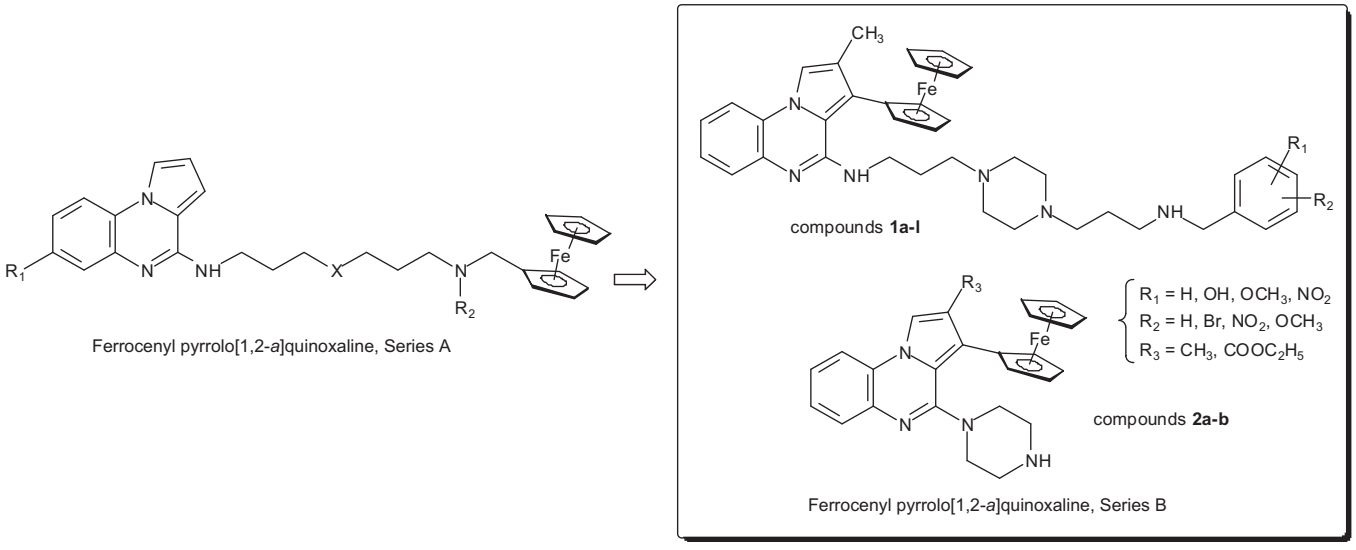


Fig. 3. General structure of new synthesized ferrocenyl pyrrolo[1,2-*a*]quinoxaline derivatives **1**–**2** (Series B).

In this report, we also synthesized two ferrocenyl pyrrolo[1,2-*a*]quinoxalinylpiperazines **2** (Fig. 3), designed as new structural analogues of previously described ferrocenic fluoroquinolones **5** (Fig. 2) [21].

Hence, we report here the synthesis and *in vitro* antiparasitic activity upon *P. falciparum* of a new series of ferrocenic pyrrolo[1,2-*a*]quinoxalines **1-2**. From biological results obtained for the new ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1-2**, preliminary structure-affinity relationships may be drawn, in relation to the chemical nature of substituents on the benzyl moiety. Finally, pharmacological results will be discussed in terms of the lipophilic behaviour of synthesized compounds quantified through the partitioning theory.

2. Chemistry

The new ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l**, and **2a-b** were both synthesized from commercially available ferrocene-carboxaldehyde. The ferrocenyl nitropropene derivative **3** was easily obtained from the ferrocene-carboxaldehyde *via* a base-catalyzed condensation of nitroethane (Scheme 1) [29]. The *E* isomer was the only product [30,31]. The ethyl 4-methyl-3-ferrocenylpyrrole-2-carboxylate **4** was synthesized using a Barton–Zard reaction [32]; the (*E*)-1-ferrocenyl-2-nitropropene **3** reacted with one equivalent of ethyl isocyanide previously anionized with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in a mixture of tetrahydrofuran and *tert*-butyl alcohol leading to pyrrole **4** [33,34]. The 3D spatial determination of this ferrocenyl-pyrrole **4** was established by X-ray crystallography, and confirmed the structure in the solid state as anticipated on the basis of IR and ^{1}H NMR data (Fig. 4). The preparation of *N*-aryl pyrrole **5** was obtained by nucleophilic substitution of the pyrrole-2-carboxylates **4** with 2-fluoro-nitrobenzene using cesium carbonate as the base in refluxing DMF solution [35–37]. Reduction of the nitro moiety of **5** with iron in hot glacial acetic acid produced the spontaneous ring closure onto the ester to afford the desired ferrocenyl pyrrolo[1,2-*a*]quinoxaline **6** through a one-pot reduction-cyclization step [36,37]. The lactam **6** was subsequently chlorodehydroxylated with phosphorous oxychloride, leading to the 4-chloroquinoxaline **7**. An X-ray single crystal analysis was also performed on pyrroloquinoxaline **7** in order to confirm the structure (Fig. 5). The structural parameters for both ferrocenyl substituents in compounds **4** and **7** are within the normal ranges, and the iron atom is sandwiched almost perfectly centrally between the two cyclopentadienyl rings. The iron distances to the two cyclopentadienyl rings do not differ in pyrrole **4** and pyrroloquinoxaline **7**. Thus, iron was observed at 1.654 (2) Å from the plane defined by the two cyclopentadienyl systems in both compounds **4** and **7**. Moreover, in the two structures (**4** and **7**), the ferrocene has adopted an eclipsed conformation.

The key intermediate **8**, used as precursor for synthesis of compounds **1**, was obtained by coupling 4-chloropyrrolo[1,2-*a*]quinoxaline **7** to *tert*-butyl *N*-[3-[4-(3-aminopropyl)piperazin-1-yl]propyl]carbamate [38,39] through a nucleophilic substitution of the chlorine atom at C-4 [18,40]. The cleavage of the Boc-protecting group of **8** was achieved using a 20% trifluoroacetic acid solution in dichloromethane leading to a mixture of trifluoroacetamide **9** and deprotected amine **10**. The trifluoroacetamide function of **9** was then easily removed with a 1 M NaOH solution in methanol to give compound **10** [41]. Various substituted benzaldehydes were then condensed with amine **10** in refluxing ethanol giving the imines **11a-l**. Reduction of **11a-l** by sodium borohydride in methanol at 0 °C gave ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** [22]. The bispyrrolo[1,2-*a*]quinoxaline **12** was synthesized by reacting 1,4-bis(3-aminopropyl)piperazine with a two equivalents amount of **7** in refluxing pentanol (Scheme 2) [22]. The 4-chloro derivatives **7** and **13** were treated with an excess of piperazine in ethylene glycol to obtain ferrocenic pyrrolo[1,2-*a*]quinoxalinylpiperazines **2a-b** (Scheme 3) [42]. The synthesis of the latter compound **13** has been also accomplished from ferrocene-carboxaldehyde according to the sequence depicted in Scheme 4. Thus, the pyrrole-diester **14** was synthesized by the reaction of two equivalents of ethyl isocyanide with ferrocene-carboxaldehyde in the presence of two equivalents of DBU [29,43]. Coupling of 2-fluoro-nitrobenzene with diethyl 3-ferrocenylpyrrole-2,4-dicarboxylate **14** in DMF at reflux in the presence of cesium carbonate afforded **15**. Reduction of the nitro function of **15** followed by intramolecular cyclization led to the pyrroloquinoxalin-6-one **16** as described above. This lactam **16** was then submitted to chlorodehydroxylation using phosphorous oxychloride to give the 4-chloroquinoxaline **13**.

3. Biological activity

3.1. In vitro antimalarial activity

All new ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1-2** were evaluated for their antimalarial activity *in vitro* upon the *P. falciparum* CQ-sensitive strain F32 (IC_{50} CQ = 0.019.5 μM) and the CQ-resistant strains FcB1 and K1 (IC_{50} CQ = 0.105 and 0.226 μM, respectively). As shown in Table 1, they were found to have IC_{50}s between 0.038 and 4.71 μM upon F32, 0.048–3.78 μM upon FcB1, and 0.060–2.86 μM upon K1 *P. falciparum* strains, respectively.

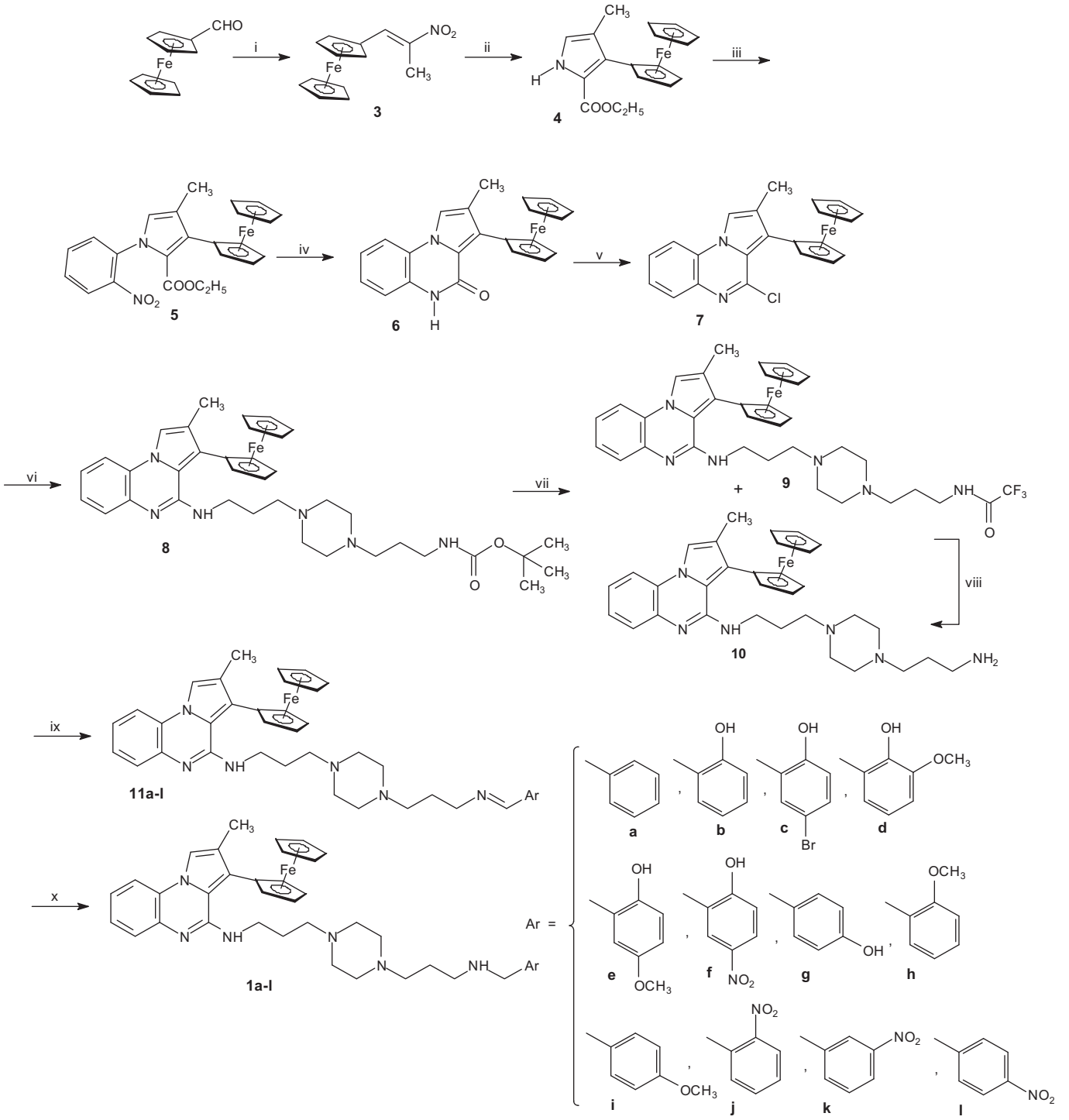
By comparing activities for compounds bearing one same type of substituent on the pyrrolo[1,2-*a*]quinoxaline moiety, it appears that compounds **1** with a benzyl substituted bis(3-aminopropyl)piperazine linker were more active (up to 4–98 times) than their counterparts with piperazine linkage (compounds **2a-b**). This indicates that the no-substituted piperazine motif, when directly attached to the pyrrolo[1,2-*a*]quinoxaline skeleton (compounds **2a-b**), does lead to less active compounds against the three *P. falciparum* strains.

As seen in Table 1, almost all compounds (except **8**, **1c**, **1g** and **2a**) have lower IC_{50} values for growth inhibition of CQ-sensitive strain F32 than for growth inhibition of CQ-resistant strains FcB1 and K1. A comparison of the IC_{50} values for the growth inhibition of the resistant between sensitive strains of *P. falciparum* suggests relatively low levels of cross-resistance to CQ. The resistance index values (Table 1), calculated from the ratio between IC_{50} for the resistant and IC_{50} for sensitive strains of *P. falciparum*, were lower than those for CQ, in a 0.2–4.5 range (FcB1) and a 0.2–3.1 range (K1), excepted for **1l** which presented a resistance index of 5.3 on FcB1, comparable to the one of CQ on the same strain (5.4).

Antimalarial activity also clearly depended on the nature of the substituent on the bis(3-aminopropyl)piperazine linker. On the three *P. falciparum* strains, the substituted benzyl bis(3-aminopropyl)piperazinyl pyrroloquinoxalines **1a-l** were found more active than the *N*-protected bis(3-aminopropyl)piperazinyl heterocyclic compounds **8** and **9**; i.e. IC_{50} = 0.048–0.949 μM for **1a-l** versus 2.45–3.72 μM for **8** or 0.99–1.12 μM for **9**. These results also demonstrated that the *N*-trifluoroacetyl protected derivative **9** was always more active than its *N*-Boc protected homologue **8**.

Among compounds **1a-l** consisting of a 1,4-bis(3-aminopropyl) piperazine linker substitued by a terminal benzyl group, the nature of this substitution led to various profiles in terms of antimalarial activity on CQ-resistant or sensible strains. Interestingly, it appears that electronic factors of the 2-hydroxybenzyl ring have minimal effect, with both electron-donating and electron-withdrawing groups giving the same range of activities. However, the 2-hydroxybenzyl substituted compound **1b** always displayed much lower activities than their methoxy, nitro or bromo substituted analogues **1c-f** (IC_{50} = 0.754 μM for **1b** versus 0.134–0.254 μM for **1c-f**).

The three pyrrolo[1,2-*a*]quinoxalines **1j-l** with nitro substitutions were found the most active compounds against the CQ-sensible F32



Scheme 1. Synthesis of new ferrocenyl pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l**; Reagents and conditions: (i) $C_2H_5NO_2$, CH_3COONH_4, Δ; (ii) CN–CH_2–$COOC_2H_5$, DBU, THF/*t*-BuOH, 50 °C; (iii) 2-fluoro-nitrobenzene, Cs_2CO_3, DMF, Δ; (iv) Fe, CH_3COOH, Δ; (v) $POCl_3$, Δ; (vi) H_2N–$(CH_2)_3$–piperazine–$(CH_2)_3$–NH-$COOC(CH_3)_3$, Δ.; (vii) CF_3COOH, CH_2Cl_2, RT; (viii) NaOH 1 M, CH_3OH/H_2O, Δ; (ix) substituted Ar-CHO, EtOH, Δ; (x) $NaBH_4$, CH_3OH, 0 °C.

strain (IC_{50} = 0.0.038–0.085 μM). The 4-methoxy substituent on the benzyl terminal group (compound **1i**) also increased the antimalarial activity against F32 strain (IC_{50} = 0.045 μM), when compared to the non-substituted counterpart **1a** (IC_{50} = 0.229 μM) or its 4-hydroxy analogue **1g** (IC_{50} = 0.186 μM). Moreover, **1i** was found 3.4 times more active than its 2-methoxy substituted analogue **1h** against F32 strain (IC_{50} = 0.156 μM). The bulky bisferrocenylpyrroloquinoxaline **12** displayed a moderate activity (IC_{50} = 0.788 μM) against the F32 strain in comparison with the monoferrocenyl pyrroloquinoxalines **1** or **10**.

Against the CQ-resistant FcB1 and K1 strains, the pyrrolo[1,2-*a*] quinoxaline **1f** bearing a 5-nitro-2-hydroxybenzyl substituent on the bis(3-aminopropyl)piperazine lateral chain exhibited the most potent antimalarial activity with IC_{50} of 0.048 and 0.060 μM, respectively on each strain. Moreover, the 5-bromo-2-hydroxybenzyl substituted derivative **1c** was also found to have

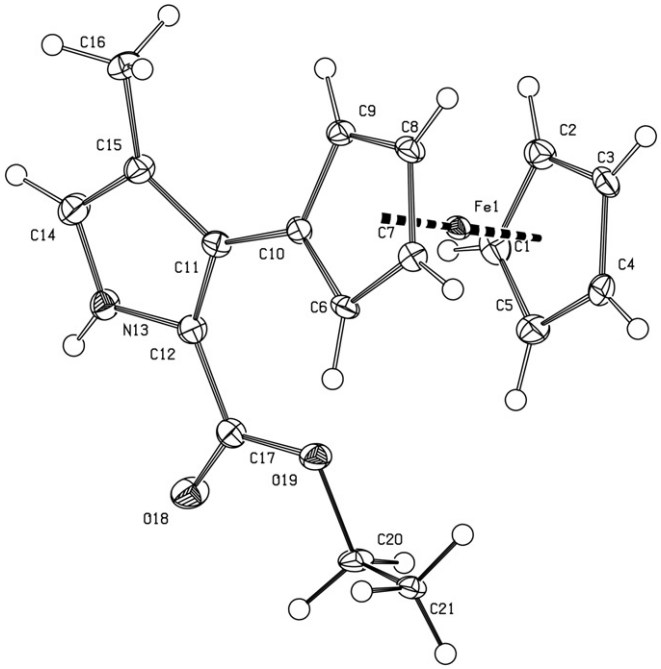


Fig. 4. The ORTEP drawing of 3-ferrocenyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester **4** with thermal ellipsoids at 30% level.

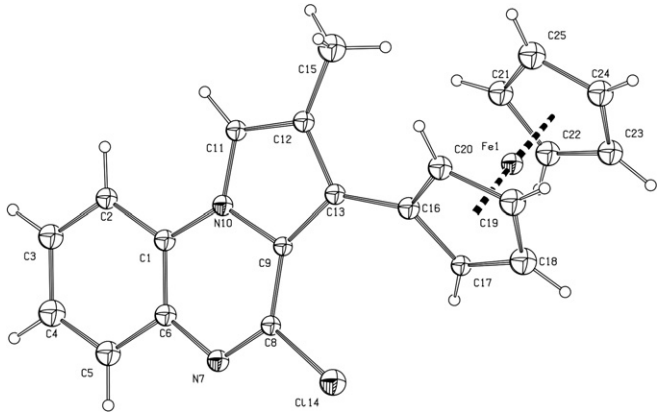


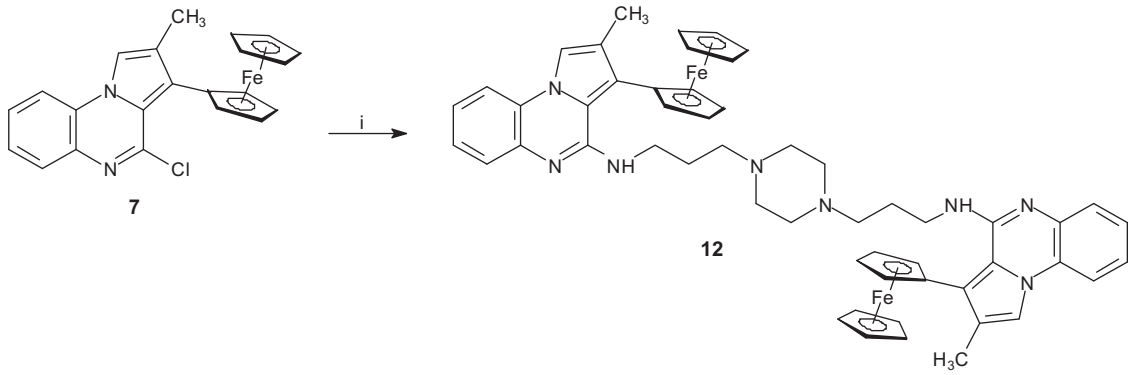
Fig. 5. The ORTEP drawing of 4-chloro-3-ferrocenyl-2-methyl-pyrrolo[1,2-*a*] quinoxaline **7** with thermal ellipsoids at 30% level.

a significant activity against the FcB1 strain (IC_{50} = 0.114 μM). From these two above data on the CQ-resistant FcB1 strain, it could be drawn that the substitution of the bis(3-aminopropyl)piperazine linker by a 2-hydroxybenzyl group with electron-withdrawing inductive effect (−NO_2 or −Br) led to a substantial increase in the antimalarial activity against these latter strain. Nevertheless, introduction of a methoxy substituent on the terminal 2-hydroxybenzyl moiety such as compounds **1d** and **1e** decreased the antiparasitic activity on FcB1 (IC_{50} = 0.341 and 0.491 μM, respectively); but this structural modification increased the potency when compared to its non-substituted counterpart **1b** (IC_{50} = 0.949 μM against FcB1 strain).

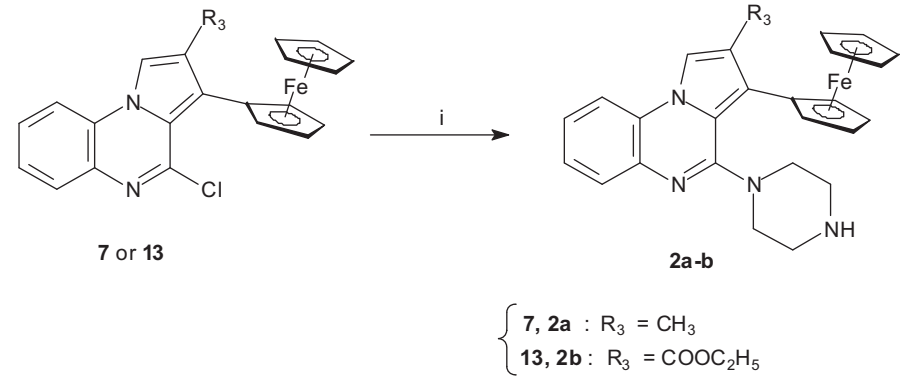
Surprisingly, it appears that a methoxy group on the benzyl moiety of the diaminoalkyl side chain (compounds **1i** and **1h**) produced the same range of activity against FcB1 parasite strain (IC_{50} = 0.166 μM). The 4-hydroxy function on this benzyl moiety (compound **1g**, IC_{50} = 0.136 μM) was noticed 6 times more potent than that with the hydroxyl group on the 2-position of this benzyl nucleus (compound **1b**, IC_{50} = 0.949 μM).

The presence of a nitro function on the benzyl ring (pyrroloquinoxalines **1j-l**) has various effect on the efficacy of these compounds in CQ-resistant FcB1 strain of *P. falciparum*; i.e. IC_{50} = 0.142 μM for **1k**, 0.213 μM for **1l** and 0.381 μM for **1j**.

Against the CQ-resistant strain (K1), compound **1i** bearing a 4-methoxybenzyl group on the lateral diaminoalkyl chain also exhibited potent antiplasmodial activity (IC_{50} = 0.089 μM). Nevertheless, its 2-methoxybenzyl analogue **1h** showed much lower antimalarial activity (IC_{50} = 0.270 μM against K1). The position of the nitro function on the benzyl (compounds **1j-l**) was less detrimental against drug-resistant strain K1: these nitro derivatives exhibited the same potency range (IC_{50} = 0.178, 0.100 and 0.123 μM, respectively). This kind of observation could be noticed for the two methoxy substituted 2-hydroxybenzyl compounds (**1d** and **1e**) which showed same level of efficacy against CQ-resistant strain K1 with IC_{50} of 0.170 μM for **1d**, and 0.175 μM for **1e**. The replacement of the nitro function in position 5 of the terminal 2-hydroxybenzyl group (compound **1f**) by a bromide atom (compound **1c**), showing a lower inductive effect, led to a decrease in the antimalarial activity; i.e. IC_{50} = 0.060 μM for **1f** versus 0.212 μM for **1c** against K1. Their unsubstituted 2-hydroxybenzyl analogue **1b** displayed an IC_{50} of 0.756 μM, whereas its 4-hydroxybenzyl homologue **1g** was found 4.7 times more active against K1 strain (IC_{50} = 0.159 μM). In addition, the primary amine intermediate 10 showed activity *in vitro* against both strains (FcB1 and K1) in the range of 0.275–0.324 μM. Its benzyl substituted analogue **1a** also showed moderate antimalarial activity with an IC_{50} of 0.330 μM against each CQ-resistant strain.



Scheme 2. Synthesis of new bis ferrocenyl pyrrolo[1,2-*a*]quinoxaline **12**; Reagents and conditions: (i) 1,4-bis(3-aminopropyl)piperazine, 1-pentanol, Δ.



Scheme 3. Synthesis of new ferrocenyl pyrrolo[1,2-*a*]quinoxaline derivatives **2a-b**; Reagents and conditions: (i) piperazine, ethylene glycol, 140 °C.

3.2. Cytotoxicity test on MRC-5 cells

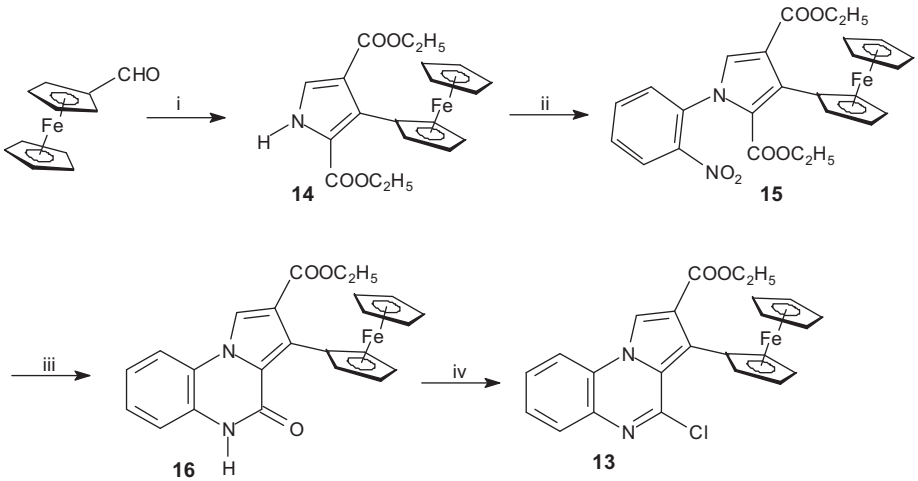
All pyrrolo[1,2-*a*]quinoxalines **1-2** showed cytotoxicity upon the MRC-5 cells in the micromolar range (IC_{50} = 2.12–9.30 μM). The different substituent modulations had less influence on the cytotoxicity IC_{50} values than that observed for the antimalarial activity. In a general way, the less cytotoxic molecules (compounds **8**–**9**, **1b**, **2a**-**b** and **12**) were the less active on the *P. falciparum* strains excepted **1j-l** that were found the most active compounds on the F32 strain. Hence, the substitution of the terminal benzylmethylamine function on the linker of compounds **1** by a nitro group (pyrroloquinoxalines **1j-l**) induced decreases in the cytotoxicity upon MRC-5 cells. Indexes of selectivity are defined as the ratio between the IC_{50} value on the MRC-5 cells and the IC_{50} value on the CQ-sensitive or CQ-resistant *P. falciparum* strains. Compounds that demonstrated high selectivity (high indexes of selectivity) should offer a potential of safer therapy. This led to identify nitro substituted compounds **1j-l** with selectivity indexes >105 upon the *P. falciparum* CQ-sensitive strain F32, that could constitute suitable candidate for further pharmacological studies.

3.3. Inhibition of β-hematin formation

In vitro experiment studies have previously established that quinoline antimalarial drugs, such as CQ, are associated with the crystallization of hemozoin [44–47]. Current evidence indicates that antimalarial drugs can act by inhibiting the formation of hemozoin, thus preventing heme detoxification. Three mechanisms of action have been proposed: (1) direct binding of the drug to heme monomers or dimers in solution which interferes with the crystallization of hemozoin [46]; (2) chemiabsorption of the drug onto crystallised hemozoin, leading to inhibition of further heme aggregation [48]; and (3) inhibition of an enzyme possibly catalyzing hemozoin crystallization [49]. The two former mechanisms can be explored through the inhibition β-hematin (the synthetic equivalent of hemozoin) formation assay. A panel of selected active compounds (**1d**, **1f-g**, **1i** and **1k-l**.) was thus tested for its ability to inhibit β-hematin formation (Table 2). On the one hand, no inhibition was observed for compound **1g**, whatever the concentration tested, and **1i** only weakly inhibited the process. On the other hand, products **1d**, **1f**, **1k** and **1l** were all able to inhibit β-hematin formation. In our hands, **1d** inhibiting activity was half of that of chloroquine; nearly twice as much compound was needed to achieve the same level of inhibition, as implied by the calculated 1.96 CQ index (Table 2). The efficiencies of **1f**, **1k** and **1l** (NO_2 substituted products) were better than that of chloroquine (CQ index below 1). Therefore, some of the *in vitro* cytocidal activity witnessed for compounds **1d**, **1f**, **1i**, **1k** and **1l** could be related to their ability to inhibit this main heme detoxification pathway of *P. falciparum*, with NO_2 substituted compounds (**1f**, **1k** and **1l**) being the most efficient.

4. Lipophilicity

Preliminary pharmacological results of tested compounds could be discussed in terms of their physicochemical behaviour through the partitioning theory, evaluated here by the distribution



Scheme 4. Synthesis of ferrocenyl pyrrolo[1,2-*a*]quinoxaline **13**; Reagents and conditions: (i) CN–CH_2–$COOC_2H_5$, DBU, THF, 50 °C; (ii) 2-fluoro-nitrobenzene, Cs_2CO_3, DMF, Δ; (iii) Fe, CH_3COOH, Δ; (iv) $POCl_3$, Δ.

Table 1
In vitro sensitivity of *P. falciparum* strains and *in vitro* cytotoxicity on MRC-5 cells.

	IC_{50} values (μM)[a]				Index of selectivity[b]			Resistance index	
	P. falciparum strains			MRC-5 cells	F32	FcB1	K1	FcB1[c]	K1[d]
Compound	F32	FcB1	K1						
CQ	0.0195 ± 0.004	0.105 ± 0.016	0.226 ± 0.052	50.00 ± 0.60	2564.1	476.2	221.2	5.4	11.6
8	3.7211 ± 0.133	2.7061 ± 0.171	2.4493 ± 0.184	6.02 ± 0.60	1.6	2.2	2.5	0.7	0.7
9	0.9890 ± 0.052	1.1228 ± 0.273	1.1233 ± 0.163	4.39 ± 0.15	4.4	3.9	3.9	1.1	1.1
10	0.1553 ± 0.034	0.2751 ± 0.042	0.3241 ± 0.032	2.12 ± 0.35	13.7	7.7	6.5	1.8	2.1
1a	0.2296 ± 0.045	0.3279 ± 0.029	0.3335 ± 0.057	2.75 ± 0.15	12.0	8.4	8.2	1.4	1.5
1b	0.7540 ± 0.047	0.9488 ± 0.102	0.7569 ± 0.023	4.47 ± 0.15	5.9	4.7	5.9	1.3	1.0
1c	0.1570 ± 0.022	0.1143 ± 0.015	0.2121 ± 0.048	2.53 ± 0.13	16.1	22.1	11.9	0.7	1.4
1d	0.1346 ± 0.034	0.3411 ± 0.038	0.1703 ± 0.019	2.00 ± 0.14	14.9	5.9	11.7	2.5	1.3
1e	0.1684 ± 0.020	0.4914 ± 0.012	0.1755 ± 0.047	3.14 ± 0.57	18.6	6.4	17.9	2.9	1.0
1f	0.2538 ± 0.056	0.0480 ± 0.014	0.0601 ± 0.011	3.49 ± 0.14	13.8	72.7	58.1	0.2	0.2
1g	0.1864 ± 0.024	0.1357 ± 0.025	0.1590 ± 0.024	2.38 ± 0.30	12.8	17.5	15.0	0.7	0.9
1h	0.1558 ± 0.028	0.1665 ± 0.041	0.2702 ± 0.124	2.19 ± 0.15	14.0	13.1	8.1	1.1	1.7
1i	0.045 ± 0.001	0.1663 ± 0.032	0.0893 ± 0.011	3.40 ± 0.20	75.6	20.4	38.1	3.7	2.0
1j	0.085 ± 0.013	0.3807 ± 0.029	0.1780 ± 0.032	9.30 ± 0.10	109.4	24.4	52.3	4.5	2.1
1k	0.038 ± 0.003	0.1423 ± 0.037	0.1003 ± 0.014	4.00 ± 0.10	105.3	28.1	39.9	3.7	2.6
1l	0.040 ± 0.009	0.2130 ± 0.011	0.1233 ± 0.003	4.80 ± 0.50	120.	22.5	38.9	5.3	3.1
2a	4.7087 ± 0.412	3.7821 ± 0.354	2.8577 ± 0.169	6.88 ± 2.22	1.5	1.8	2.4	0.8	0.6
2b	2.30 ± 0.404	4.90 ± 1.90	3.70 ± 0.50	8.90 ± 0.30	3.9	1.8	2.4	2.1	1.6
12	0.7881 ± 0.043	0.8534 ± 0.108	0.8376 ± 0.058	6.03 ± 0.11	7.7	7.1	7.2	1.1	1.1

[a] IC_{50} values were measured on the chloroquine-sensitive strain F32/Tanzania and the chloroquine-resistant strains FcB1/Colombia and K1/Thailand. The IC_{50} (μM) values correspond to the mean +/− standard deviation from 3 independent experiments.
[b] Index of selectivity (I.S.) was defined as the ratio between the IC_{50} value on the MRC-5 cells and the IC_{50} value against the *P. falciparum* F32, FcB1 and K1 strains.
[c] IC_{50} (FcB1)/IC_{50} (F32).
[d] IC_{50} (K1)/IC_{50} (F32).

coefficient *D* (usually expressed as log *D*) [50]. Consequently, HPLC determination of log *D* was achieved for ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1**–**2**, **8**–**10** and **12** at two distinct pHs (5.0 considered close to the probable pH of the digestive vacuole, and 7.4 assumed to be the cytosol pH). A plot of antimalarial IC_{50} versus log *D* values at pH = 7.4 and 5.0 was presented in Figs. 6 and 7, respectively, permitting to classify compounds in various subsets. At pH 7.4, all of studied compounds were found very lipophilic with log *D* values between 2.84 for **10** and 4.69 for **8**. The most active ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives with a benzyl substituted bis(3-aminopropyl)piperazine linker (**1a-l**) have log *D* values between 3.79 and 4.55. Nevertheless, in this series a group of four compounds (**1**a-**b**, **1e** and **9**) with log *D* values in the 4.19–4.37 range showed more moderate activities. A heterogenous behaviour was found for less active compounds **2a-b** and **12**, with log *D* between 3.46 and 3.84. In this last case, introduction of a piperazine moiety on the ferrocenic pyrrolo[1,2-*a*]quinoxaline nucleus considerably decreases antimalarial activities. Such an observation in the decrease of the antiparasitic activity could also be noticed for lipophilic compound **8** with log *D* of 4.69.

At pH 5.0, the most active compounds (**1a-l**, **9**–**10** and **12**) were found the less lipophilic with log *D* values inferior to 1.73. Nevertheless, three compounds in this series (derivatives **1b**, **9** and **12** at log *D* = 0.57, 0.95 and 0.41 respectively) were found slightly less active than the other ones. Conversely, the less active three compounds on *P. falciparum* strains (**2a-b** and **8**) are more lipophilic with log *D* between 2.12 and 2.89.

These results clearly indicate that the choice and the substitution of the terminal benzyl in the bis(3-aminopropyl)piperazine linker of the ferrocenyl pyrrolo[1,2-*a*]quinoxaline moiety correlated with a particular lipophilic behaviour seem to be fundamental for the antimalarial activity observed for this new series.

Table 2
Haem polymerisation inhibitory activity of active compounds **1d**, **1f-g**, **1i** and **1k-l**.

Compound	Concentration (mM)	Inhibition (%)[a]	IC_{50} (mM)[a]	CQ index[b]
CQ	1	48.6 ± 17.08		
	2	74.7 ± 14.53	1.02 ± 0.19	1
1d	2	49.9 ± 15.84	~2	~1.96
1f	1	58.0 ± 19.16		
	2	84.6 ± 12.42	0.88 ± 0.13	0.86
1g	0.1–2	0	–	–
1i	2	23.6 ± 17.48	>2	>1.96
1k	1	79.7 ± 18.61		
	2	82.2 ± 13.31	0.64 ± 0.15	0.62
1l	1	59.0 ± 22.87		
	2	74.6 ± 24.94	0.62 ± 0.24	0.61

[a] Mean ± standard deviation from three independent experiments.
[b] IC_{50} compound/IC_{50} chloroquine.

5. Conclusion

In conclusion, novel series of ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives **1-2** were synthesized from ferrocene-carboxaldehyde and tested for their *in vitro* activity upon the erythrocytic development of *P. falciparum* strains with different chloroquine-resistance status. Promising pharmacological results were obtained, leading to the selection of nitro substituted compounds **1j-l** as the most potent candidates. However, the targeted design of intramolecular hydrogen bonds on the diaminoalkyl linker of our ferrocenic heterocyclic compounds did not lead to detrimental antimalarial activities in comparison with our derivatives which did not contain an intramolecular hydrogen-bonding motif. Nevertheless, these new ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives could open the way to original valuable medicinal chemistry scaffolding. Furthermore, this work contributes to validate the choice of the ferrocene moiety as a template useful for designing new antimalarial compounds.

6. Experimental

6.1. Chemistry

Commercialy reagents were used as received without additional purification. Melting points were determinated with an SM-LUX-POL Leitz hot-stage microscope and are uncorrected. IR spectra were recorded on a BRUKER IFS-25 spectrophotometer. NMR

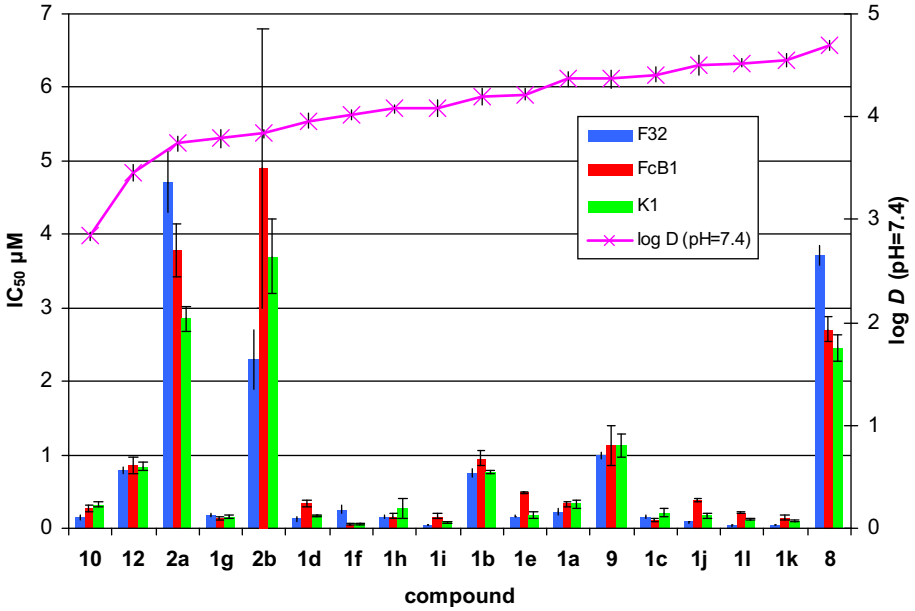


Fig. 6. Log *D*/activity relationship for pyrrolo[1,2-*a*]quinoxalines **1**–**2**, **8**–**10** and **12** at pH = 7.4.

spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE 300 spectrometer (^{1}H), a BRUKER AVANCE II/200 spectrometer (^{13}C), and a BRUKER AVANCE III/600 spectrometer (2D-COSY, HSQC and HMBC). Splitting patterns have been designated as follows: s = singlet; bs = broad singlet; d = doublet; t = triplet; q = quartet; qt = quintuplet; m = multiplet. Analytical TLC was carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV$_{254}$) with visualisation by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography. Mass spectra were recorded on a Micromass-Waters Q-TOF Ultima spectrometer. Elemental analyses (C, H, N) for new compounds were performed by CNRS (Vernaison-France) and agreed with the proposed structures within ±0.3% of the theoretical values.

*6.1.1. (E)-1-Ferrocenyl-2-nitropropene (**3**)*

To a solution of ferrocene-carboxaldehyde (23.36 mmol, 1 equiv) in 80 mL of nitroethane, was added ammonium acetate (23.36 mmol, 1 equiv). The reaction mixture was heated under reflux for 6 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was extracted with ethyl acetate (180 mL). The organic layer was washed with water (2 × 40 mL), then with brine (30 mL), dried over Na_2SO_4, and the solvent was evaporated to dryness. The residue was purified by

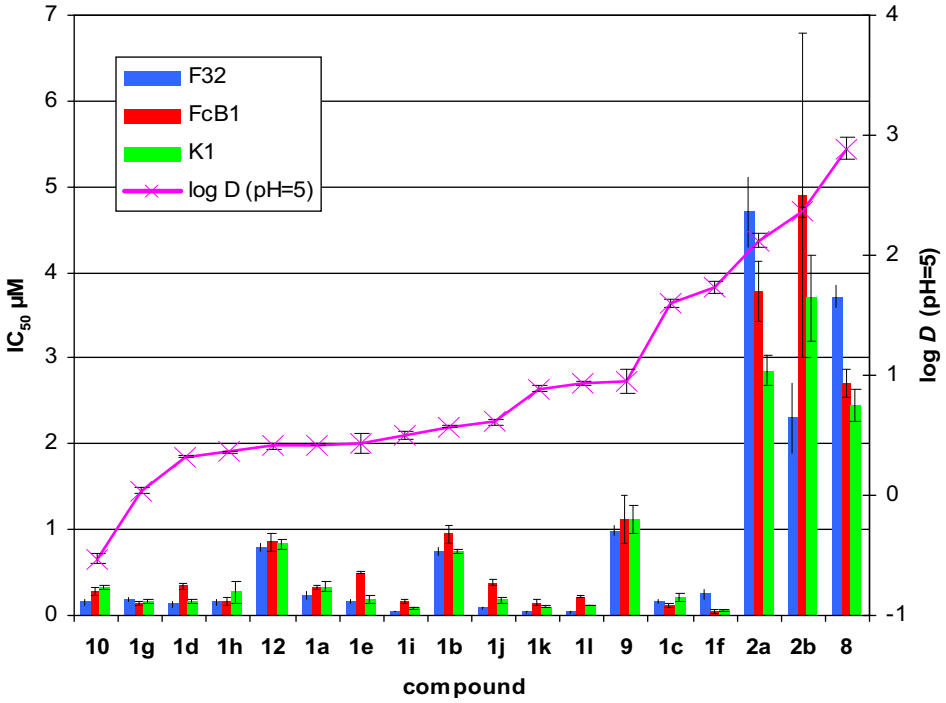


Fig. 7. Log *D*/activity relationship for pyrrolo[1,2-*a*]quinoxalines **1**–**2**, **8**–**10** and **12** at pH = 5.0.

column chromatography using AcOEt/cyclohexane (30/70 − *v/v*) as eluent to yield **3**. Red oil (98%). ^{1}H NMR ($CDCl_3$) δ: 7.95 (s, 1H, H-1), 4.55 (s, 4H, Cp-ortho and Cp-meta), 4.21 (s, 5H, Cp′), 2.32 (s, 3H, CH_3). Anal. Calcd. for $C_{13}H_{13}NO_2Fe$: C, 57.60; H, 4.83; N, 5.17. Found: C, 57.46; H, 5.02; N, 5.30.

*6.1.2. Ethyl 3-ferrocenyl-4-methyl-1H-pyrrole-2-carboxylate (**4**)*

To a solution of (*E*)-1-ferrocenyl-2-nitropropene **3** (23 mmol, 1 equiv) in 40 mL of tetrahydrofuran and 20 mL of *tert*-butyl alcohol, were added DBU (25.3 mmol, 1.1 equiv) and ethyl isocyanide (34.5 mmol, 1.5 equiv). The mixture was heated for 4 h at 50 °C. Concentration of the solvents gave an oil which was washed with water. The residue was taken up in dichloromethane. The organic layer was dried over Na_2SO_4, and removed under reduced pressure. The solid residue was then purified by column chromatography using CH_2Cl_2/cyclohexane (80/20 − *v/v*) as eluent to yield **4**. Orange crystals (79%); mp 108 °C. IR (KBr) 3330 (NH), 1690 (C=O). ^{1}H NMR ($CDCl_3$) δ: 8.90 (bs, 1H, NH), 6.70 (s, 1H, H-5), 4.81 (s, 2H, Cp-ortho), 4.33 (s, 2H, Cp-meta), 4.29 (q, 2H, J = 7.20 Hz, CH_2), 4.12 (s, 5H, Cp′), 2.37 (s, 3H, CH_3), 1.34 (t, 3H, J = 7.20 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 162.1 (C=O), 128.2 (C-2), 122.7 (C-5), 122.1 (C-3), 120.4 (C-4), 80.7 (Cp-1), 71.4 (Cp-ortho), 70.7 (Cp′), 69.0 (Cp-meta), 61.5 (CH_2), 15.9 (CH_3), 14.0 (CH_3). Anal. Calcd. for $C_{18}H_{19}NO_2Fe$: C, 64.12; H, 5.68; N, 4.15. Found: C, 64.35; H, 5.47; N, 4.11.

*6.1.3. Ethyl 3-ferrocenyl-4-methyl-1-(2-nitrophenyl)pyrrole-2-carboxylate (**5**)*

To a solution of ethyl 3-ferrocenyl-4-methylpyrrole-2-carboxylate **4** (17.4 mmol, 1 equiv) in 55 mL of DMF was added 1-fluoro-2-nitrobenzene (26.1 mmol, 1.5 equiv) and cesium carbonate (20.9 mmol, 1.2 equiv). The reaction mixture was refluxed for 1 h 30 min, then diluted in AcOEt (220 mL) after cooling. The reaction mixture was filtered, washed with water (2 × 200 mL), then brine (200 mL) and dried over sodium sulphate. The organic layer was concentrated under vacuo to give an orange oil which was purified by column chromatography using AcOEt/cyclohexane (20/80 − *v/v*) as eluent to yield **5**. Orange oil (87%). IR (KBr) 1695 (C=O). ^{1}H NMR ($CDCl_3$) δ: 8.04 (d, 1H, J = 8.10 Hz, H-3′), 7.66 (t, 1H, J = 8.10 Hz, H-5′), 7.54 (t, 1H, J = 8.10 Hz, H-4′), 7.37n (d, 1H, J = 8.10 Hz, H-6′), 6.65 (s, 1H, H-5), 4.74 (s, 2H, Cp-ortho), 4.36 (s, 2H, Cp-meta), 4.19 (s, 5H, Cp′), 3.96 (q, 2H, J = 7.20 Hz, CH_2), 2.40 (s, 3H, CH_3), 0.95 (t, 3H, J = 7.20 Hz, CH_3). Anal. Calcd. for $C_{24}H_{22}N_2O_4Fe$: C, 62.90; H, 4.84; N, 6.11. Found: C, 63.07; H, 4.73; N, 5.98.

*6.1.4. 3-Ferrocenyl-2-methyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (**6**)*

A suspension of **5** (15.1 mmol, 1 equiv) and iron powder (60.4 mmol, 4 equiv) in 65 mL of acetic acid was heated under reflux for 1 h 30 min. The reaction mixture was cooled, suspended in 200 mL of a 1 M aqueous solution of HCl, agitated, then filtered off, washed with HCl 1 M (100 mL), water, Et_2O and dried to give **6**. Orange crystals (76%); mp >260 °C. IR (KBr) 3200, 2750 (NH), 1660 (C=O). ^{1}H NMR (DMSO-d_6) δ: 10.99 (s, 1H, NH), 8.03 (s, 1H, H-1), 7.92 (d, 1H, J = 7.80 Hz, H-9), 7.22 (d, 1H, J = 7.80 Hz, H-6), 7.19–7.14 (m, 2H, H-7 and H-8), 4.95 (s, 2H, Cp-ortho), 4.31 (s, 2H, Cp-meta), 4.07 (s, 5H, Cp′), 2.48 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 155.1 (C=O), 128.2 (C-5a), 125.0 (C-6), 124.5 (C-9a), 122.6 (C-3a), 122.3 (C-7), 122.2 (C-8), 118.3 (C-3), 117.3 (C-2), 115.6 (C-9), 114.3 (C-1), 78.7 (Cp-1), 70.4 (Cp-ortho), 69.0 (Cp′), 67.2 (Cp-meta), 12.8 (CH_3). Anal. Calcd. for $C_{22}H_{18}N_2OFe$: C, 69.13; H, 4.75; N, 7.33. Found: C, 69.27; H, 4.53; N, 7.06.

*6.1.5. 4-Chloro-3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxaline (**7**)*

A solution of 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one **6** (11 mmol) in $POCl_3$ (35 mL) was refluxed for 4 h. After removing excess of reactive under *vacuum*, the residue was carefully dissolved in water at 0 °C and the resulting solution was made basic with 32% aqueous ammonium hydroxide solution. The precipitate was filtered, washed with water, dried and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate and evaporated to dryness. The crude residue was then purified by column chromatography using dichloromethane as eluent to give **6**. Red crystals (75%); mp 178 °C. ^{1}H NMR ($CDCl_3$) δ: 7.82 (dd, 1H, J = 8.20 and 1.30 Hz, H-9), 7.81 (s, 1H, H-1), 7.74 (dd, 1H, J = 8.20 and 1.30 Hz, H-6), 7.49 (ddd, 1H, J = 8.20, 7.20 and 1.30 Hz, H-8), 7.39 (ddd, 1H, J = 8.20, 7.20 and 1.30 Hz, H-7), 4.70 (s, 2H, Cp-ortho), 4.41 (s, 2H, Cp-meta), 4.18 (s, 5H, Cp′), 2.72 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 145.6 (C-4), 135.9 (C-5a), 130.3 (C-6), 129.4 (C-7), 128.2 (C-9a), 127.0 (C-3a), 126.7 (C-8), 122.3 (C-3), 121.4 (C-2), 116.9 (C-9), 114.7 (C-1), 82.2 (Cp-1), 73.5 (Cp-ortho), 71.4 (Cp′), 69.2 (Cp-meta), 14.1 (CH_3). Anal. Calcd. for $C_{22}H_{17}ClN_2Fe$: C, 65.95; H, 4.28; N, 6.99. Found: C, 66.12; H, 4.48; N, 7.09.

*6.1.6. tert-Butyl-3-{4-[3-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)aminopropyl]piperazin-1-yl}propylcarbamate (**8**)*

A mixture of 4-chloro-3-ferrocenyl-2-methylpyrrolo[1,2-*a*]quinoxaline **7** (4.5 mmol, 1 equiv) and *tert*-butyl *N*-[3-[4-(3-aminopropyl)piperazin-1-yl]propyl]carbamate (20.25 mmol, 4.5 equiv) was heated initially at 90 °C for 1 h 30 min with stirring, and subsequently at 110 °C for 3 h 30 min with continued stirring to drive the reaction to completion. After cooling to room temperature, the resulting brownish oil was then suspended in ice, triturated and recovered by filtration. The crude precipitate was then washed with water and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and evaporated to dryness. The crude residue was then purified by column chromatography using dichloromethane/methanol (90/10 − *v/v*) as eluent to yield **8**. Orange crystals (82%); mp 66 °C. IR (KBr) 3420 and 3345 (NH), 1705 (C=O). ^{1}H NMR ($CDCl_3$) δ: 7.62 (s, 1H, H-1′), 7.62 (dd, 1H, J = 8.10 and 1.20 Hz, H-9′), 7.56 (dd, 1H, J = 8.10 and 1.20 Hz, H-6′), 7.26 (ddd, 1H, J = 8.10, 7.20 and 1.20 Hz, H-8′), 7.16 (ddd, 1H, J = 8.10, 7.20 and 1.20 Hz, H-7′), 6.19 (t, 1H, J = 4.80 Hz, NH), 5.45 (bs, 1H, NH carbamate), 4.53 (t, 2H, J = 1.75 Hz, Cp-ortho), 4.42 (t, 2H, J = 1.75 Hz, Cp-meta), 4.23 (s, 5H, Cp′), 3.73–3.65 (m, 2H, NCH_2), 3.24–3.18 (m, 2H, NCH_2), 2.60–2.47 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.91–1.85 (qt, 2H, J = 7.20 Hz, CH_2), 1.73–1.68 (qt, 2H, J = 7.20 Hz, CH_2), 1.45 (s, 9H, CH_3). Anal. Calcd. for $C_{37}H_{48}N_6O_2Fe$: C, 66.86; H, 7.28; N, 12.64. Found: C, 66.98; H, 7.04; N, 12.82.

*6.1.7. General procedure for 2,2,2-trifluoro-N-(3-{4-[3-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)aminopropyl]piperazin-1-yl}propyl)acetamide (**9**) and 1-{N-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(3-aminopropyl)piperazine (**10**)*

To a solution of **8** (3.48 mmol) in 27 mL of dichloromethane was added 40 mL of a 20% trifluoroacetic acid solution in dichloromethane. The mixture was stirred at room temperature for 14 h, then neutralized with 65 mL of a saturated aqueous solution of potassium carbonate and extracted with 3 × 70 mL of dichloromethane. The organic layer was washed with water, then brine and dried with anhydrous sodium sulphate. The solvent was removed under reduced pressure. The crude residue was then purified by column chromatography using dichloromethane/methanol/NH_4OH (80/20/1 − *v/v/v*) as eluent to give compounds **9** and **10**.

*6.1.7.1. 2,2,2-Trifluoro-N-(3-{4-[3-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)aminopropyl]piperazin-1-yl}propyl)acetamide (**9**).* Orange crystals (18%); R_f = 0.80; mp 65 °C. IR (KBr) 3400 and 3240 (NH), 1685 (C=O). ^{1}H NMR ($CDCl_3$) δ: 9.63 (s, 1H, $NHCOCF_3$), 7.61–7.56 (m, 3H, H-1′, H-9′ and H-6′), 7.26 (t, 1H, J = 7.50 Hz, H-8′), 7.15 (t, 1H, J = 7.50 Hz, H-7′), 6.17 (t, 1H, J = 4.80 Hz, NH), 4.53 (t, 2H, J = 1.60 Hz, Cp-ortho), 4.40 (t, 2H, J = 1.60 Hz, Cp-meta), 4.21 (s, 5H,

Cp′), 3.72–3.65 (m, 2H, NCH_2), 3.49–3.46 (m, 2H, NCH_2), 2.60–2.41 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.90–1.81 (m, 2H, CH_2), 1.78–1.73 (m, 2H, CH_2). Anal. Calcd. for $C_{34}H_{39}F_3N_6OFe$: C, 61.82; H, 5.95; N, 12.72. Found: C, 61.92; H, 5.81; N, 12.93. HRMS $[M + H]^+$ $C_{34}H_{39}F_3N_6OFe$ requires 661.2565, found 661.2533.

*6.1.7.2. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(3-aminopropyl)piperazine (**10**).* Orange crystals (59%); $R_f = 0.15$; mp 109 °C. IR (KBr) 3350 and 3275 (NH_2 and NH). ^1H NMR ($CDCl_3$) δ: 7.58 (s, 1H, H-1′), 7.57 (dd, 1H, J = 8.40 and 1.20 Hz, H-9′), 7.53 (dd, 1H, J = 8.40 and 1.20 Hz, H-6′), 7.23 (ddd, 1H, J = 8.40, 7.20 and 1.20 Hz, H-8′), 7.13 (ddd, 1H, J = 8.40, 7.20 and 1.20 Hz, H-7′), 6.15 (t, 1H, J = 4.75 Hz, NH), 4.50 (t, 2H, J = 1.60 Hz, Cp-ortho), 4.38 (t, 2H, J = 1.60 Hz, Cp-meta), 4.20 (s, 5H, Cp′), 3.68–3.61 (m, 2H, NCH_2), 3.27 (bs, 2H, NH_2), 2.85–2.81 (m, 2H, NCH_2), 2.53–2.37 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.86–1.77 (qt, 2H, J = 7.10 Hz, CH_2), 1.71–1.66 (qt, 2H, J = 7.10 Hz, CH_2). ^{13}C NMR ($CDCl_3$) δ: 151.0 (C-4′), 138.2 (C-5a′), 127.6 (C-6′), 126.3 (C-9a′), 126.1 (C-7′), 124.7 (C-3a′), 123.7 (C-8′), 117.8 (C-3′), 116.2 (C-2′), 115.0 (C-9′), 114.2 (C-1′), 83.2 (Cp-1), 71.3 (Cp-ortho), 71.0 (Cp′), 69.4 (Cp-meta), 59.6 (NCH_2), 57.4 (NCH_2), 54.5 (CH_2 pip.), 43.1 (NCH_2), 40.3 (NCH_2), 28.1 (CH_2), 24.5 (CH_2), 14.0 (CH_3). Anal. Calcd. for $C_{32}H_{40}N_6Fe$: C, 68.08; H, 7.14; N, 14.89. Found: C, 67.92; H, 7.23; N, 15.06.

*6.1.8. General procedure for 1-{N-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(N-arylmethylene-3-aminopropyl)piperazine (**11a-l**)*

To a solution of compound **10** (0.36 mmol) in ethanol (7 mL) was added substituted benzaldehyde (0.36 mmol, 1 equiv). The reaction mixture was then refluxed for 4 h. The mixture was evaporated to dryness under reduced pressure. After cooling, the residue was extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The oily residue was used without further purification.

*6.1.8.1. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(N-phenylmethylene-3-aminopropyl)piperazine (**11a**).* Orange crystals (87%); mp 78 °C. IR (KBr) 3270 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 8.28 (s, 1H, N=CH), 7.87 (d, 1H, J = 7.75 Hz, H-9′), 7.73–7.70 (m, 2H, H-1′ and H-6′), 7.60–7.53 (m, 3H, H-2″, H-4″ and H-6″), 7.43–7.40 (m, 2H, H-3″ and H-5″), 7.24 (t, 1H, J = 7.75 Hz, H-8′), 7.17 (t, 1H, J = 7.75 Hz, H-7′), 6.20 (t, 1H, J = 4.70 Hz, NH), 4.50 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.40 (t, 2H, J = 1.65 Hz, Cp-meta), 4.19 (s, 5H, Cp′), 3.68–3.65 (m, 4H, NCH_2), 2.75–2.53 (m, 15H, NCH_2, CH_2 pip. and CH_3), 2.04–2.01 (m, 2H, CH_2), 1.93–1.90 (m, 2H, CH_2). Anal. Calcd. for $C_{39}H_{44}N_6Fe$: C, 71.77; H, 6.80; N, 12.88. Found: C, 71.65; H, 6.89; N, 12.76.

*6.1.8.2. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxyphenylmethylene)-3-aminopropyl] piperazine (**11b**).* Orange crystals (74%); mp 91 °C. IR (KBr) 3500 (OH), 3260 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 8.36 (s, 1H, N=CH), 7.59 (s, 1H, H-1′), 7.58 (d, 1H, J = 7.90 Hz, H-9′), 7.54 (d, 1H, J = 7.90 Hz, H-6′), 7.29 (d, 1H, J = 7.40 Hz, H-6″), 7.24–7.22 (m, 2H, H-5″ and H-8′), 7.14 (*t*, 1H, J = 7.90 Hz, H-7′), 7.00 (d, 1H, J = 7.40 Hz, H-3″), 6.86 (t, 1H, J = 7.40 Hz, H-4″), 6.18 (t, 1H, J = 4.75 Hz, NH), 4.51 (t, 2H, J = 1.70 Hz, Cp-ortho), 4.40 (t, 2H, J = 1.70 Hz, Cp-meta), 4.20 (s, 5H, Cp′), 3.69–3.61 (m, 4H, NCH_2), 2.61–2.51 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.95–1.86 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{44}N_6OFe$: C, 70.05; H, 6.63; N, 12.57. Found: C, 69.87; H, 6.51; N, 12.70.

*6.1.8.3. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(5-bromo-2-hydroxyphenylmethylene)-3-aminopropyl]piperazine (**11c**).* Orange crystals (83%); mp 85 °C. IR (KBr) 3510 (OH), 3250 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 13.35 (bs, 1H, OH), 8.30 (s, 1H, N=CH), 7.69–7.60 (m, 3H, H-1′, H-6′ and H-9′), 7.39–7.22 (m, 4H, H-7′, H-8′, H-4″ and H-6″), 6.85 (d, 1H, J = 7.50 Hz, H-3″), 6.54 (bs, 1H, NH), 4.51 (t, 2H, J = 1.50 Hz, Cp-ortho), 4.48 (t, 2H, J = 1.50 Hz, Cp-meta), 4.23 (s, 5H, Cp′), 3.82–3.80 (m, 2H, NCH_2), 3.71–3.68 (m, 2H, NCH_2), 2.95–2.56 (m, 15H, NCH_2, CH_2 pip. and CH_3), 2.12–1.96 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{43}BrN_6OFe$: C, 62.66; H, 5.80; N, 11.24. Found: C, 62.77; H, 5.62; N, 11.07.

*6.1.8.4. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-3-methoxyphenylmethylene)-3-aminopropyl]piperazine (**11d**).* Orange crystals (91%); mp 89 °C. IR (KBr) 3520 (OH), 3250 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 13.90 (bs, 1H, OH), 8.36 (s, 1H, N=CH), 7.65–7.62 (m, 3H, H-1′, H-6′ and H-9′), 7.25–7.17 (m, 2H, H-7′ and H-8′), 6.97–6.76 (m, 3H, H-4″, H-5″ and H-6″), 6.42 (t, 1H, J = 4.50 Hz, NH), 4.52 (t, 2H, J = 1.60 Hz, Cp-ortho), 4.46 (t, 2H, J = 1.60 Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.91 (s, 3H, CH_3O), 3.80–3.70 (m, 4H, NCH_2), 2.96–2.53 (m, 15H, NCH_2, CH_2 pip. and CH_3), 2.18–1.93 (m, 4H, CH_2). Anal. Calcd. for $C_{40}H_{46}N_6O_2Fe$: C, 68.76; H, 6.64; N, 12.03. Found: C, 68.54; H, 6.50; N, 11.92.

*6.1.8.5. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-5-methoxyphenylmethylene)-3-aminopropyl]piperazine (**11e**).* Orange crystals (84%); mp 59 °C. IR (KBr) 3500 (OH), 3250 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 12.91 (bs, 1H, OH), 8.34 (s, 1H, N=CH), 7.63 (s, 1H, H-1′), 7.61 (d, 1H, J = 7.60 Hz, H-9′), 7.57 (d, 1H, J = 7.60 Hz, H-6′), 7.26 (t, 1H, J = 7.60 Hz, H-8′), 7.17 (t, 1H, J = 7.60 Hz, H-7′), 7.02–6.78 (m, 3H, H-3″, H-4″ and H-6″), 6.25 (t, 1H, J = 4.55 Hz, NH), 4.53 (t, 2H, J = 1.60 Hz, Cp-ortho), 4.43 (t, 2H, J = 1.60 Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.79 (s, 3H, CH_3O), 3.70–3.65 (m, 4H, NCH_2), 2.72–2.56 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.99–1.95 (m, 4H, CH_2). Anal. Calcd. for $C_{40}H_{46}N_6O_2Fe$: C, 68.76; H, 6.64; N, 12.03. Found: C, 68.92; H, 6.76; N, 11.85.

*6.1.8.6. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-5-nitrophenylmethylene)-3-aminopropyl]piperazine (**11f**).* Orange crystals (95%); mp 105 °C. IR (KBr) 3500 (OH), 3260 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 14.52 (bs, 1H, OH), 8.25 (s, 1H, N=CH), 8.15 (d, 1H, J = 1.0 Hz, H-6″), 8.04 (dd, 1H, J = 8.70 and 1.0 Hz, H-4″), 7.58–7.52 (m, 3H, H-1′, H-9′ and H-6′), 7.28–7.14 (m, 2H, H-8′ and H-7′), 6.75 (d, 1H, J = 8.70 Hz, H-3″), 6.30 (t, 1H, J = =4.55 Hz, NH), 4.48 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.40 (t, 2H, J = 1.65 Hz, Cp-meta), 4.18 (s, 5H, Cp′), 3.70–3.67 (m, 4H, NCH_2), 2.84–2.51 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.99–1.90 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{43}N_7O_3Fe$: C, 65.64; H, 6.07; N, 13.74. Found: C, 65.73; H, 6.24; N, 13.97.

*6.1.8.7. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-hydroxyphenylmethylene)-3-aminopropyl] piperazine (**11g**).* Orange crystals (92%); mp 82 °C. IR (KBr) 3510 (OH), 3260 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 8.11 (s, 1H, N=CH), 7.69–7.56 (m, 5H, H-1′, H-9′, H-6′, H-2″ and H-6″), 7.26 (t, 1H, J = 7.50 Hz, H-8′), 7.17 (t, 1H, J = 7.50 Hz, H-7′), 6.87 (d, 2H, = 8.10 Hz, H-3″ and H-5″), 6.20 (t, 1H, J = 4.50 Hz, NH), 4.50 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.41 (t, 2H, J = 1.65 Hz, Cp-meta), 4.20 (s, 5H, Cp′), 3.65–3.58 (m, 4H, NCH_2), 2.67–2.52 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.96–1.85 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{44}N_6OFe$: C, 70.05; H, 6.63; N, 12.57. Found: C, 69.83; H, 6.87; N, 12.41.

*6.1.8.8. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-methoxyphenylmethylene)-3-aminopropyl] piperazine (**11h**).* Orange crystals (74%); mp 82 °C. IR (KBr) 3270 (NH), 1620 (C=N). ^1H NMR ($CDCl_3$) δ: 8.73 (s, 1H, N=CH), 7.93 (dd, 1H, J = 7.50 and 1.50 Hz, H-6″), 7.60 (s, 1H, H-1′), 7.59 (d, 1H, J = 7.75 Hz, H-9′), 7.55 (d, 1H, J = 7.75 Hz, H-6′), 7.39 (ddd, 1H, J = 8.40, 7.50 and 1.50 Hz, H-5″), 7.25 (t, 1H, J = 7.75 Hz, H-8′), 7.15 (t,

1H, J = 7.75 Hz, H-7′), 7.02–6.97 (m, 1H, H-4″), 6.92 (d, 1H, J = 8.40 Hz, H-3″), 6.17 (t, 1H, J = 4.50 Hz, NH), 4.53 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.42 (t, 2H, J = 1.65 Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.88 (s, 3H, CH_3O), 3.70–3.65 (m, 4H, NCH_2), 2.61–2.46 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.98–1.90 (m, 2H, CH_2), 1.88–1.85 (m, 2H, CH_2). Anal. Calcd. for $C_{40}H_{46}N_6OFe$: C, 70.37; H, 6.79; N, 12.31. Found: C, 70.30; H, 6.96; N, 12.09.

*6.1.8.9. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-methoxyphenylmethylene)-3-aminopropyl] piperazine (**11i**).* Orange crystals (97%); mp 62 °C. IR (KBr) 3280 (NH), 1615 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.19 (s, 1H, N=CH), 7.65 (d, 2H, J = 8.10 Hz, H-2″ and H-6″), 7.58–7.53 (m, 3H, H-1′, H-9′ and H-6′), 7.25 (t, 1H, J = 7.60 Hz, H-8′), 7.12 (t, 1H, J = 7.60 Hz, H-7′), 6.91 (d, 2H, J = 8.10 Hz, H-3″ and H-5″), 6.17 (t, 1H, J = 4.60 Hz, NH), 4.49 (t, 2H, J = 1.60 Hz, Cp-ortho), 4.38 (t, 2H, J = 1.60 Hz, Cp-meta), 4.18 (s, 5H, Cp′), 3.81 (s, 3H, CH_3O), 3.68–3.60 (m, 4H, NCH_2), 2.64–2.46 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.96–1.86 (m, 4H, CH_2). Anal. Calcd. for $C_{40}H_{46}N_6OFe$: C, 70.37; H, 6.79; N, 12.31. Found: C, 70.55; H, 6.87; N, 12.39.

*6.1.8.10. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-nitrophenylmethylene)-3-aminopropyl] piperazine (**11j**).* Orange crystals (84%); mp 84 °C. IR (KBr) 3285 (NH), 1620 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.66 (s, 1H, N=CH), 8.00–7.95 (m, 2H, H-3″ and H-6″), 7.78–7.52 (m, 5H, H-1′, H-9′, H-6′, H-4″ and H-5″), 7.24 (t, 1H, J = 7.50 Hz, H-8′), 7.16 (t, 1H, J = 7.50 Hz, H-7′), 6.32 (t, 1H, J = 4.55 Hz, NH), 4.49 (t, 2H, J = 1.55 Hz, Cp-ortho), 4.43 (t, 2H, J = 1.55 Hz, Cp-meta), 4.19 (s, 5H, Cp′), 3.73–3.69 (m, 4H, NCH_2), 2.92–2.75 (m, 12H, NCH_2 and CH_2 pip.), 2.53 (s, 3H, CH_3), 2.06–1.96 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{43}N_7O_2Fe$: C, 67.14; H, 6.21; N, 14.05. Found: C, 67.38; H, 6.05; N, 13.89.

*6.1.8.11. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(3-nitrophenylmethylene)-3-aminopropyl] piperazine (**11k**).* Orange crystals (96%); mp 101 °C. IR (KBr) 3300 (NH), 1615 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.57 (d, 1H, J = 1.20 Hz, H-2″), 8.38 (s, 1H, N=CH), 8.28 (ddd, 1H, J = 8.30, 1.20 and 1.20 Hz, H-4″), 8.06 (dd, 1H, J = 8.30 and 1.20 Hz, H-6″), 7.65–7.58 (m, 4H, H-1′, H-9′, H-6′ and H-5″), 7.25 (t, 1H, J = 7.20 Hz, H-8′), 7.21 (t, 1H, J = 7.20 Hz, H-7′), 6.43 (bs, 1H, NH), 4.50 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.46 (t, 2H, J = 1.65 Hz, Cp-meta), 4.23 (s, 5H, Cp′), 3.77–3.71 (m, 4H, NCH_2), 2.95–2.85 (m, 12H, NCH_2 and CH_2 pip.), 2.57 (s, 3H, CH_3), 2.08–1.98 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{43}N_7O_2Fe$: C, 67.14; H, 6.21; N, 14.05. Found: C, 67.02; H, 6.46; N, 13.80.

*6.1.8.12. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-nitrophenylmethylene)-3-aminopropyl] piperazine (**11l**).* Orange crystals (85%); mp 98 °C. IR (KBr) 3305 (NH), 1615 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.39 (s, 1H, N=CH), 8.28 (d, 2H, J = 8.55 Hz, H-3″ and H-5″), 7.90 (d, 2H, J = 8.55 Hz, H-2″ and H-6″), 7.74–7.61 (m, 3H, H-1′, H-9′ and H-6′), 7.29–7.15 (m, 2H, H-8′ and H-7′), 6.49 (bs, 1H, NH), 4.51 (t, 2H, J = 1.65 Hz, Cp-ortho), 4.48 (t, 2H, J = 1.65 Hz, Cp-meta), 4.24 (s, 5H, Cp′), 3.79–3.72 (m, 4H, NCH_2), 3.18–2.74 (m, 12H, NCH_2 and CH_2 pip.), 2.57 (s, 3H, CH_3), 2.16–1.89 (m, 4H, CH_2). Anal. Calcd. for $C_{39}H_{43}N_7O_2Fe$: C, 67.14; H, 6.21; N, 14.05. Found: C, 67.36; H, 6.11; N, 13.87.

*6.1.9. General procedure for 1-{N-(3-ferrocenyl-2-methylpyrrolo [1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(N-benzyl-3-aminopropyl)piperazine (**1a-l**)*

To a solution of compound **11a-l** (6.0 mmol) in a mixture of methanol (10 mL) and tetrahydrofuran (4 mL) at 0 °C was added $NaBH_4$ (12 mmol, 2 equiv) in small portions. After the mixture was stirred at 0 °C for 1.5 h, the reaction was quenched by addition of 30 mL of water. Then the mixture was extracted with dichloromethane (2 × 30 mL). The organic layers were washed with brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude residue was then purified by column chromatography using chloroform/methanol (70/30 – *v/v*) as eluent to give compounds **1a-l**.

*6.1.9.1. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-(N-benzyl-3-aminopropyl)piperazine (**1a**).* Orange crystals (42%); mp 50 °C. R_f = 0.11. IR (KBr) 3420 and 3350 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.61 (s, 1H, H-1′), 7.60 (dd, 1H, J = 8.20 and 1.35 Hz, H-9′), 7.57(dd, 1H, J = 8.20 and 1.35 Hz, H-6′), 7.37–7.35 (m, 3H, H-2″, H-4″ and H-6″), 7.32–7.28 (m, 2H, H-3″ and H-5″), 7.23 (ddd, 1H, J = 8.20, 7.50 and 1.35 Hz, H-8′), 7.16 (ddd, 1H, J = 8.20, 7.50 and 1.35 Hz, H-7′), 6.17 (t, 1H, J = 4.80 Hz, NH), 4.53 (t, 2H, J = 1.80 Hz, Cp-ortho), 4.41 (t, 2H, J = 1.80 Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.83 (s, 2H, NCH_2), 3.71–3.64 (m, 2H, NCH_2), 2.75 (t, 2H, J = 6.60 Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.52–2.38 (m, 12H, NCH_2 and CH_2 pip.), 1.86–1.75 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.9 (C-4′), 138.2 (C-5a′), 130.2 (C-1″), 129.9 (C-2″ and C-6″), 129.5 (C-3″ and C-5″), 128.7 (C-4″), 127.4 (C-6′), 126.2 (C-9a′), 126.0 (C-7′), 124.6 (C-3a′), 123.6 (C-8′), 117.7 (C-3′), 116.1 (C-2′), 114.9 (C-9′), 114.2 (C-1′), 83.0 (Cp-1), 71.2 (Cp-ortho), 70.9 (Cp′), 69.3 (Cp-meta), 58.5 (NCH_2), 57.6 (NCH_2), 57.6 (NCH_2), 54.9 (NCH_2), 54.6 (CH_2 pip.), 54.5 (CH_2 pip.), 49.6 (NCH_2), 40.3 (NCH_2), 28.0 (CH_2), 27.4 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{39}H_{46}N_6Fe$: C, 71.55; H, 7.08; N, 12.84. Found: C, 71.59; H, 7.22; N, 12.68.

*6.1.9.2. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxybenzyl)-3-aminopropyl]piperazine (**1b**).* Orange crystals (62%); mp 66 °C. R_f = 0.11. IR (KBr) 3510 (OH), 3420 and 3360 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.62 (s, 1H, H-1′), 7.61 (d, 1H, J = 7.80 Hz, H-9′), 7.56(d, 1H, J = 7.80 Hz, H-6′), 7.27–7.14 (m, 3H, H-7′, H-8′ and H-5″), 7.04–7.01 (m, 1H, H-4″), 6.96–6.88 (m, 1H, H-6″), 6.80–6.77 (m, 1H, H-3″), 6.18 (t, 1H, J = 4.50 Hz, NH), 4.53 (t, 2H, J = 1.50 Hz, Cp-ortho), 4.42 (t, 2H, J = 1.50 Hz, Cp-meta), 4.23 (s, 5H, Cp′), 4.01 (s, 2H, NCH_2), 3.69–3.67 (m, 2H, NCH_2), 2.81–2.78 (m, 2H, NCH_2), 2.58–2.44 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.88–1.78 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 159.6 (C-2″), 151.0 (C-4′), 138.1 (C-5a′), 130.2 (C-6″), 129.1 (C-4″), 127.4 (C-6′), 126.3 (C-7′), 124.8 (C-9a′), 123.8 (C-1″), 123.5 (C-3a′), 122.0 (C-8′), 120.5 (C-5″), 117.8 (C-3′), 116.4 (C-2′), 115.1 (C-9′), 115.0 (C-1′), 114.3 (C-3″), 83.1 (Cp-1), 71.3 (Cp-ortho), 71.0 (Cp′), 69.4 (Cp-meta), 58.3 (NCH_2), 57.3 (NCH_2), 54.4 (2 CH_2 pip.), 53.6 (NCH_2), 49.2 (NCH_2), 40.4 (NCH_2), 27.9 (CH_2), 27.0 (CH_2), 14.0 (CH_3). Anal. Calcd. for $C_{39}H_{46}N_6OFe$: C, 69.84; H, 6.91; N, 12.53. Found: C, 69.98; H, 7.23; N, 12.37.

*6.1.9.3. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(5-bromo-2-hydroxybenzyl)-3-aminopropyl] piperazine (**1c**).* Orange crystals (76%); mp 63 °C. R_f = 0.11. IR (KBr) 3500 (OH), 3420 and 3350 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.61 (s, 1H, H-1′), 7.60 (d, 1H, J = 7.80 Hz, H-9′), 7.56(d, 1H, J = 7.80 Hz, H-6′), 7.26 (t, 1H, J = 7.80 Hz, H-8′), 7.23 (s, 1H, H-6″), 7.16 (d, 1H, J = 7.50 Hz, H-4″), 7.10 (t, 1H, J = 7.80 Hz, H-7′), 6.71 (d, 1H, J = 7.50 Hz, H-3″), 6.18 (t, 1H, J = 4.55 Hz, NH), 4.53 (t, 2H, J = 1.55 Hz, Cp-ortho), 4.40 (t, 2H, J = 1.55 Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.94 (s, 2H, NCH_2), 3.70–3.66 (m, 2H, NCH_2), 2.73–2.71 (m, 2H, NCH_2), 2.55 (s, 3H, CH_3), 2.50–2.40 (m, 12H, NCH_2 and CH_2 pip.), 1.87–1.82 (m, 2H, CH_2), 1.75–1.71 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 158.8 (C-2″), 150.9 (C-4′), 138.0 (C-5a′), 132.7 (C-6″), 132.4 (C-4″), 127.3 (C-6′), 126.2 (C-3a′), 126.0 (C-7′), 125.6 (C-1″), 124.6 (C-9a′), 123.7 (C-8′), 119.5 (C-3″), 117.6 (C-3′), 116.2 (C-2′), 114.9 (C-9′), 114.2 (C-1′), 111.9 (C-5″), 83.0 (Cp-1), 71.2 (Cp-ortho), 70.9 (Cp′), 69.3 (Cp-meta), 58.2 (NCH_2), 57.4 (2 NCH_2), 54.4 (2 CH_2 pip.), 49.1 (NCH_2), 40.3 (NCH_2), 27.9 (CH_2), 27.0 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{39}H_{45}BrN_6OFe$: C, 62.49; H, 6.05; N, 11.21. Found: C, 62.64; H, 5.87; N, 11.30.

*6.1.9.4. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-3-methoxybenzyl)-3-aminopropyl] piperazine (**1d**).* Orange crystals (76%); mp 63 °C. $R_f = 0.11$. IR (KBr) 3485 (OH), 3420 and 3355 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.62 (s, 1H, H-1′), 7.61 (dd, 1H, $J = 8.10$ and 1.20 Hz, H-9′), 7.56 (dd, 1H, $J = 8.10$ and 1.20 Hz, H-6′), 7.26 (ddd, 1H, $J = 8.10$, 7.60 and 1.20 Hz, H-8′), 7.18 (ddd, 1H, $J = 8.10$, 7.60 and 1.20 Hz, H-7′), 6.81 (dd, 1H, $J = 7.50$ and 1.65 Hz, H-6″), 6.77 (t, 1H, $J = 7.50$ Hz, H-5″), 6.71 (dd, 1H, $J = 7.50$ and 1.65 Hz, H-4″), 6.18 (t, 1H, $J = 4.65$ Hz, NH), 4.53 (t, 2H, $J = 1.55$ Hz, Cp-ortho), 4.42 (t, 2H, $J = 1.55$ Hz, Cp-meta), 4.23 (s, 5H, Cp′), 4.04 (s, 2H, NCH_2), 3.87 (s, 3H, CH_3O), 3.72–3.62 (m, 2H, NCH_2), 2.83 (t, 2H, $J = 6.00$ Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.51–2.36 (m, 12H, NCH_2 and CH_2 pip.), 1.86–1.79 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.7 (C-4′), 149.2 (C-3″), 148.6 (C-2″), 138.0 (C-5a′), 127.3 (C-6′), 126.0 (C-9a′), 125.9 (C-7′), 124.5 (C-1″), 123.8 (C-3a′), 123.2 (C-8′), 121.8 (C-3′), 119.8 (C-6″), 117.5 (C-5″), 116.0 (C-2′), 114.8 (C-9′), 114.0 (C-1′), 112.1 (C-4″), 82.8 (Cp-1), 71.0 (Cp-ortho), 70.7 (Cp′), 69.1 (Cp-meta), 58.2 (NCH_2), 57.3 (NCH_2), 57.1 (NCH_2), 54.4 (CH_2 pip. and CH_3O), 53.2 (CH_2 pip.), 49.0 (NCH_2), 40.1 (NCH_2), 27.8 (CH_2), 27.1 (CH_2), 13.7 (CH_3). Anal. Calcd. for $C_{40}H_{48}N_6O_2Fe$: C, 68.57; H, 6.90; N, 11.99. Found: C, 68.74; H, 7.09; N, 12.21.

*6.1.9.5. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-5-methoxybenzyl)-3-aminopropyl] piperazine (**1e**).* Orange crystals (55%); mp 63 °C. $R_f = 0.27$. IR (KBr) 3490 (OH), 3420 and 3360 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.63 (s, 1H, H-1′), 7.61 (d, 1H, $J = 7.80$ Hz, H-9′), 7.57 (d, 1H, $J = 7.80$ Hz, H-6′), 7.26 (t, 1H, $J = 7.80$ Hz, H-8′), 7.20 (t, 1H, $J = 7.80$ Hz, H-7′), 6.99–6.61 (m, 3H, H-3″, H-4″ and H-6″), 6.27 (t, 1H, $J = 4.50$ Hz, NH), 4.53 (t, 2H, $J = 1.80$ Hz, Cp-ortho), 4.44 (t, 2H, $J = 1.80$ Hz, Cp-meta), 4.23 (s, 5H, Cp′), 3.99 (s, 2H, NCH_2), 3.74 (s, 3H, CH_3O), 3.71–3.68 (m, 2H, NCH_2), 2.85 (t, 2H, $J = 6.10$ Hz, NCH_2), 2.63–2.50 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.92–1.85 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 153.8 (C-5″), 153.3 (C-2″), 150.9 (C-4′), 138.1 (C-5a′), 127.4 (C-6′), 126.3 (C-9a′), 126.1 (C-7′), 124.7 (C-1″), 124.3 (C-3a′), 123.7 (C-8′), 118.1 (C-3′), 117.7 (C-2′), 116.2 (C-9′), 115.8 (C-6″), 115.0 (C-4″), 114.9 (C-1′), 114.2 (C-3″), 83.0 (Cp-1), 71.2 (Cp-ortho), 70.9 (Cp′), 69.3 (Cp-meta), 58.2 (NCH_2), 57.5 (NCH_2), 57.2 (NCH_2), 54.5 (CH_2 pip. and CH_3O), 53.7 (CH_2 pip.), 49.1 (NCH_2), 40.3 (NCH_2), 28.0 (CH_2), 27.2 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{40}H_{48}N_6O_2Fe$: C, 68.57; H, 6.90; N, 11.99. Found: C, 68.68; H, 7.01; N, 12.07.

*6.1.9.6. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-hydroxy-5-nitrobenzyl)-3-aminopropyl] piperazine (**1f**).* Orange crystals (68%); mp 95 °C. $R_f = 0.28$. IR (KBr) 3530 (OH), 3420 and 3345 (NH). ^{1}H NMR ($CDCl_3$) δ: 8.05 (d, 1H, $J = 8.25$ Hz, H-4″), 7.91 (s, 1H, H-6″), 7.60 (d, 1H, $J = 8.05$ Hz, H-9′), 7.59 (s, 1H, H-1′), 7.54 (d, 1H, $J = 8.05$ Hz, H-6′), 7.25 (t, 1H, $J = 8.05$ Hz, H-8′), 7.15 (t, 1H, $J = 8.05$ Hz, H-7′), 6.91 (bs, 2H, NH and OH),6.78 (d, 1H, $J = 8.25$ Hz, H3″), 6.18 (t, 1H, $J = 4.80$ Hz, NH), 4.51 (t, 2H, $J = 1.65$ Hz, Cp-ortho), 4.39 (t, 2H, $J = 1.65$ Hz, Cp-meta), 4.21 (s, 5H, Cp′), 4.02 (s, 2H, NCH_2), 3.70–3.65 (m, 2H, NCH_2), 2.77 (t, 2H, $J = 6.85$ Hz, NCH_2), 2.54 (s, 3H, CH_3), 2.48–2.36 (m, 12H, NCH_2 and CH_2 pip.), 1.84–1.82 (m, 2H, CH_2), 1.76–1.73 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.9 (C-4′), 139.9 (C-2″), 137.6 (C-5a′), 127.1 (C-4″), 126.3 (C-6′), 126.2 (C-5″), 126.1 (C-7′), 124.9 (C-9a′), 124.0 (C-6″), 123.1 (C-8′), 122.3 (C-3a′), 119.8 (C-3′), 118.6 (C-3″), 117.3 (C-1″), 116.7 (C-2′), 115.2 (C-9′), 114.3 (C-1′), 82.8 (Cp-1), 71.2 (Cp-ortho), 70.9 (Cp′), 69.5 (Cp-meta), 58.3 (NCH_2), 57.1 (NCH_2), 54.1 (CH_2 pip.), 53.9 (CH_2 pip.), 49.3 (NCH_2), 48.7 (NCH_2), 40.1 (NCH_2), 27.8 (CH_2), 27.7 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{39}H_{45}N_7O_3Fe$: C, 65.45; H, 6.34; N, 13.70. Found: C, 65.64; H, 6.21; N, 13.95.

*6.1.9.7. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-hydroxybenzyl)-3-aminopropyl]piperazine (**1g**).* Orange crystals (70%); mp 69 °C. $R_f = 0.14$. IR (KBr) 3510 (OH), 3430 and 3350 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.64–7.60 (m, 3H, H-1′, H-9′ and H-6′), 7.25 (ddd, 1H, $J = 8.10$, 7.20 and 1.40 Hz, H-8′), 7.17 (ddd, 1H, $J = 8.10$, 7.20 and 1.40 Hz, H-7′), 7.13 (d, 2H, $J = 8.10$ Hz, H-2″ and H-6″), 6.70 (d, 2H, $J = 8.10$ Hz, H-3″ and H-5″), 6.18 (t, 1H, $J = 4.75$ Hz, NH), 4.51 (t, 2H, $J = 1.80$ Hz, Cp-ortho), 4.40 (t, 2H, $J = 1.80$ Hz, Cp-meta), 4.21 (s, 5H, Cp′), 3.71 (s, 2H, NCH_2), 3.66–3.59 (m, 2H, NCH_2), 2.78 (t, 2H, $J = 6.70$ Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.47–2.36 (m, 12H, NCH_2 and CH_2 pip.), 1.79–1.72 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 157.9 (C-4″), 151.1 (C-4′), 137.9 (C-5a′), 131.4 (C-1″), 131.1 (C-2″ and C-6″), 127.2 (C-6′), 126.3 (C-9a′), 126.2 (C-7′), 124.8 (C-3a′), 123.8 (C-8′), 117.7 (C-3′), 117.3 (C-3″ and C-5″), 116.5 (C-2′), 115.1 (C-9′), 114.3 (C-1′), 83.0 (Cp-1), 71.3 (Cp-ortho), 70.9 (Cp′), 69.4 (Cp-meta), 58.6 (NCH_2), 58.1 (NCH_2), 54.7 (NCH_2), 54.5 (CH_2 pip.), 54.3 (CH_2 pip.), 49.6 (NCH_2), 40.5 (NCH_2), 27.9 (CH_2), 27.4 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{39}H_{46}N_6OFe$: C, 69.84; H, 6.91; N, 12.53. Found: C, 70.03; H, 7.10; N, 12.46.

*6.1.9.8. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-methoxybenzyl)-3-aminopropyl]piperazine (**1h**).* Orange crystals (49%); mp 53 °C. $R_f = 0.35$. IR (KBr) 3400 and 3340 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.60 (s, 1H, H-1′), 7.59 (d, 1H, $J = 7.90$ Hz, H-9′), 7.56 (d, 1H, $J = 7.90$ Hz, H-6′), 7.28–7.23 (m, 3H, H-8′, H-5″ and H-6″), 7.16 (t, 1H, $J = 7.90$ Hz, H-7′), 6.95–9.93 (m, 1H, H-4″), 6.87 (d, 1H, $J = 8.10$ Hz, H-3″), 6.16 (t, 1H, $J = 4.80$ Hz, NH), 4.53 (t, 2H, $J = 1.65$ Hz, Cp-ortho), 4.41 (t, 2H, $J = 1.65$ Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.85 (s, 3H, CH_3O), 3.83 (s, 2H, NCH_2), 3.69–3.66 (m, 2H, NCH_2), 2.71 (t, 2H, $J = 6.60$ Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.53–2.38 (m, 12H, NCH_2 and CH_2 pip.), 1.87–1.73 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 158.9 (C-2″), 150.8 (C-4′), 138.1 (C-5a′), 131.8 (C-6″), 130.4 (C-4″), 127.3 (C-6′), 126.9 (C-9a′), 126.2 (C-7′), 125.9 (C-1″), 124.6 (C-3a′), 123.6 (C-8′), 122.0 (C-5″), 117.6 (C-3′), 116.1 (C-2′), 114.8 (C-9′), 114.1 (C-1′), 111.7 (C-3″), 82.9 (Cp-1), 71.1 (Cp-ortho), 70.8 (Cp′), 69.2 (Cp-meta), 58.5 (NCH_2), 57.4 (NCH_2), 56.7 (NCH_2), 54.4 (2 CH_2 pip.), 49.4 (NCH_2), 40.2 (NCH_2), 27.9 (CH_2), 26.5 (CH_2), 13.8 (CH_3). Anal. Calcd. for $C_{40}H_{48}N_6OFe$: C, 70.17; H, 7.07; N, 12.27. Found: C, 70.37; H, 7.23; N, 12.11.

*6.1.9.9. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-methoxybenzyl)-3-aminopropyl]piperazine (**1i**).* Orange crystals (80%); mp 60 °C. $R_f = 0.46$. IR (KBr) 3420 and 3340 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.61 (s, 1H, H-1′), 7.60 (dd, 1H, $J = 8.10$ and 1.50 Hz, H-9′), 7.56 (dd, 1H, $J = 8.10$ and 1.50 Hz, H-6′), 7.27 (d, 2H, $J = 7.80$ Hz, H-2″ and H-6″), 7.24 (ddd, 1H, $J = 8.10$, 7.20 and 1.50 Hz, H-8′), 7.15 (ddd, 1H, $J = 8.10$, 7.20 and 1.50 Hz, H-7′), 6.88 (d, 2H, $J = 7.80$ Hz, H-3″ and H-5″), 6.16 (t, 1H, $J = 4.80$ Hz, NH), 4.53 (t, 2H, $J = 1.50$ Hz, Cp-ortho), 4.41 (t, 2H, $J = 1.50$ Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.81 (s, 3H, CH_3O), 3.76 (s, 2H, NCH_2), 3.69–3.66 (m, 2H, NCH_2), 3.07 (bs, 1H, NH), 2.72 (t, 2H, $J = 6.60$ Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.46–2.38 (m, 12H, NCH_2 and CH_2 pip.), 1.87–1.80 (m, 2H, CH_2), 1.78–1.73 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 160.0 (C-4″), 150.9 (C-4′), 138.1 (C-5a′), 133.6 (C-1″), 130.7 (C-2″ and C-6″), 127.4 (C-6′), 126.2 (C-9a′), 126.0 (C-7′), 124.6 (C-3a′), 123.6 (C-8′), 117.6 (C-3′), 116.1 (C-2′), 115.1 (C-3″ and C-5″), 114.8 (C-9′), 114.1 (C-1′), 83.0 (Cp-1), 71.2 (Cp-ortho), 70.8 (Cp′), 69.2 (Cp-meta), 58.4 (NCH_2), 57.6 (NCH_2), 56.6 (NCH_2), 54.6 (2 CH_2 pip.), 49.3 (NCH_2), 40.3 (NCH_2), 28.1 (CH_2), 28.0 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{40}H_{48}N_6OFe$: C, 70.17; H, 7.07; N, 12.27. Found: C, 70.03; H, 7.24; N, 12.08.

*6.1.9.10. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(2-nitrobenzyl)-3-aminopropyl]piperazine (**1j**).* Orange crystals (55%); mp 58 °C. $R_f = 0.60$. IR (KBr) 3420 and 3345 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.97 (dd, 1H, $J = 8.10$ and 1.40 Hz, H-3″), 7.70 (dd, 1H, $J = 8.10$ and 1.40 Hz, H-6″), 7.64–7.58 (m, 3H, H-1′, H-9′ and H-4″), 7.56 (dd, 1H, $J = 8.10$ and 1.50 Hz, H-6′), 7.43 (ddd,

1H, $J = 8.10$, 7.20 and 1.40 Hz, H-5″), 7.25 (ddd, 1H, $J = 8.10$, 7.20 and 1.50 Hz, H-8′), 7.13 (ddd, 1H, $J = 8.10$, 7.20 and 1.50 Hz, H-7′), 6.15 (t, 1H, $J = 5.25$ Hz, NH), 4.53 (t, 2H, $J = 1.85$ Hz, Cp-ortho), 4.41 (t, 2H, $J = 1.85$ Hz, Cp-meta), 4.22 (s, 5H, Cp′), 4.07 (s, 2H, NCH_2), 3.69–3.63 (m, 2H, NCH_2), 2.79 (t, 2H, $J = 6.45$ Hz, NCH_2), 2.56 (s, 3H, CH_3), 2.51–2.35 (m, 12H, NCH_2 and CH_2 pip.), 1.85–1.76 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.8 (C-2″), 150.2 (C-4′), 138.0 (C-5″), 136.4 (C-5a′), 134.5 (C-4″), 132.7 (C-1″), 129.3 (C-3″), 127.3 (C-6′), 126.1 (C-9a′), 125.9 (C-7′ and C-6″), 124.5 (C-3a′), 123.5 (C-8′), 117.5 (C-3′), 116.0 (C-2′), 114.8 (C-9′), 114.1 (C-1′), 82.9 (Cp-1), 71.1 (Cp-ortho), 70.7 (Cp′), 69.2 (Cp-meta), 58.1 (NCH_2), 57.4 (NCH_2), 54.4 (2 CH_2 pip.), 51.9 (NCH_2), 49.6 (NCH_2), 40.2 (NCH_2), 27.8 (CH_2), 27.7 (CH_2), 13.8 (CH_3). Anal. Calcd. for $C_{39}H_{45}N_7O_2Fe$: C, 66.95; H, 6.48; N, 14.01. Found: C, 67.12; H, 6.33; N, 13.86.

*6.1.9.11. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(3-nitrobenzyl)-3-aminopropyl]piperazine (**1k**).* Orange crystals (58%); mp 65 °C. $R_f = 0.60$. IR (KBr) 3420 and 3285 (NH). ^1H NMR ($CDCl_3$) δ: 8.23 (s, 1H, H-2″), 8.12 (d, 1H, $J = 7.95$ Hz, H-4″), 7.71 (d, 1H, $J = 7.95$ Hz, H-6″), 7.61–7.48 (m, 4H, H-1′, H-9′, H-6′ and H-5″), 7.26 (t, 1H, $J = 7.60$ Hz, H-8′), 7.16 (t, 1H, $J = 7.60$ Hz, H-7′), 6.18 (t, 1H, $J = 5.10$ Hz, NH), 4.53 (t, 2H, $J = 1.75$ Hz, Cp-ortho), 4.41 (t, 2H, $J = 1.75$ Hz, Cp-meta), 4.22 (s, 5H, Cp′), 3.92 (s, 2H, NCH_2), 3.70–3.67 (m, 2H, NCH_2), 2.74 (t, 2H, $J = 6.30$ Hz, NCH_2), 2.56–2.42 (m, 15H, NCH_2, CH_2 pip. and CH_3), 1.88–1.76 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.7 (C-3″), 149.5 (C-4′), 143.9 (C-1″), 138.0 (C-5a′), 135.4 (C-6″), 130.4 (C-5″), 127.3 (C-6′), 126.1 (C-9a′), 125.9 (C-7′), 124.5 (C-3a′), 123.9 (C-2″), 123.5 (C-8′), 123.1 (C-4″), 117.5 (C-3′), 116.0 (C-2′), 114.8 (C-9′), 114.0 (C-1′), 82.9 (Cp-1), 71.1 (Cp-ortho), 70.7 (Cp′), 69.2 (Cp-meta), 58.3 (NCH_2), 57.4 (NCH_2), 54.5 (2 CH_2 pip.), 54.2 (NCH_2), 49.5 (NCH_2), 40.2 (NCH_2), 28.0 (CH_2), 27.8 (CH_2), 13.8 (CH_3). Anal. Calcd. for $C_{39}H_{45}N_7O_2Fe$: C, 66.95; H, 6.48; N, 14.01. Found: C, 66.84; H, 6.58; N, 13.93.

*6.1.9.12. 1-{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}-4-[N-(4-nitrobenzyl)-3-aminopropyl]piperazine (**1l**).* Orange crystals (56%); mp 60 °C. $R_f = 0.72$. IR (KBr) 3420 and 3350 (NH). ^1H NMR ($CDCl_3$) δ: 8.16 (d, 2H, $J = 8.85$ Hz, H-3″ and H-5″), 7.59 (dd, 1H, $J = 8.10$ and 1.45 Hz, H-9′), 7.58 (s, 1H, H-1′), 7.55 (dd, 1H, $J = 8.10$ and 1.45 Hz, H-6′), 7.48 (d, 2H, $J = 8.85$ Hz, H-2″ and H-6″), 7.23 (ddd, 1H, $J = 8.10$, 7.20 and 1.45 Hz, H-8′), 7.14 (ddd, 1H, $J = 8.10$, 7.20 and 1.45 Hz, H-7′), 6.16 (t, 1H, $J = 5.25$ Hz, NH), 4.51 (t, 2H, $J = 1.80$ Hz, Cp-ortho), 4.39 (t, 2H, $J = 1.80$ Hz, Cp-meta), 4.20 (s, 5H, Cp′), 3.87 (s, 2H, NCH_2), 3.70–3.63 (m, 2H, NCH_2), 2.68 (t, 2H, $J = 6.60$ Hz, NCH_2), 2.53 (s, 3H, CH_3), 2.52–2.39 (m, 12H, NCH_2 and CH_2 pip.), 1.89–1.80 (m, 2H, CH_2), 1.78–1.68 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.8 (C-4″), 149.4 (C-1″), 148.1 (C-4′), 138.0 (C-5a′), 129.8 (C-2″ and C-6″), 127.2 (C-6′), 126.1 (C-9a′), 125.9 (C-7′), 124.8 (C-3″ and C-5″), 124.5 (C-3a′), 123.5 (C-8′), 117.4 (C-3′), 116.0 (C-2′), 114.8 (C-9′), 114.1 (C-1′), 82.9 (Cp-1), 71.1 (Cp-ortho), 70.7 (Cp′), 69.2 (Cp-meta), 58.1 (NCH_2), 57.4 (NCH_2), 54.4 (2 CH_2 pip.), 54.3 (NCH_2), 49.4 (NCH_2), 40.2 (NCH_2), 27.9 (CH_2), 27.8 (CH_2), 13.8 (CH_3). Anal. Calcd. for $C_{39}H_{45}N_7O_2Fe$: C, 66.95; H, 6.48; N, 14.01. Found: C, 67.17; H, 6.32; N, 13.79.

*6.1.10. Bis{N-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)-3-aminopropyl}piperazine (**12**)*

A solution of 4-chloro-3-ferrocenyl-2-methylpyrrolo[1,2-*a*]quinoxaline **7** (1 mmol, 2 equiv) and 1,4-bis(3-aminopropyl)piperazine (0.5 mmol, 1 equiv) in 7 mL of 1-pentanol was heated and held at reflux (160 °C) for 24 h. After cooling to room temperature, the mixture was diluted with 15 mL of CH_2Cl_2. The organic layer was washed with 10% NaOH (3 × 8 mL), then with 8 mL of water, and dried over Na_2SO_4. The solvents were evaporated, and the residue was purified by flash column chromatography using CH_2Cl_2/methanol/NH_4OH (80/20/1 − *v/v/v*) as eluent to afford **12**. Orange oil (46%). $R_f = 0.57$. IR (KBr) 3350 (NH). ^1H NMR ($CDCl_3$) δ: 7.58 (s, 2H, H-1′), 7.56 (dd, 2H, $J = 8.40$ and 1.20 Hz, H-9′), 7.53 (dd, 2H, $J = 8.40$ and 1.20 Hz, H-6′), 7.24 (ddd, 2H, $J = 8.40$, 7.30 and 1.20 Hz, H-8′), 7.15 (ddd, 2H, $J = 8.40$, 7.30 and 1.20 Hz, H-7′), 6.15 (t, 2H, $J = 4.50$ Hz, NH), 4.53 (t, 4H, $J = 1.50$ Hz, Cp-ortho), 4.40 (t, 4H, $J = 1.50$ Hz, Cp-meta), 4.20 (s, 10H, Cp′), 3.69–3.63 (m, 4H, NCH_2), 3.05–3.03 (m, 4H, NCH_2), 2.58–2.43 (m, 14H, CH_2 pip. and CH_3), 1.86–1.79 (m, 4H, CH_2), 1.78–1.68 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 150.9 (C-4′), 138.1 (C-5a′), 127.4 (C-6′), 126.2 (C-9a′), 126.1 (C-7′), 124.7 (C-3a′), 123.8 (C-8′), 117.6 (C-3′), 116.2 (C-2′), 115.0 (C-9′), 114.2 (C-1′), 83.0 (Cp-1), 71.3 (Cp-ortho), 70.9 (Cp′), 69.3 (Cp-meta), 57.5 (NCH_2), 54.6 (NCH_2), 52.8 (CH_2 pip.), 45.8 (NCH_2), 40.1 (NCH_2), 27.6 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{54}H_{56}N_8Fe_2$: C, 69.83; H, 6.08; N, 12.06. Found: C, 70.07; H, 6.12; N, 12.21.

*6.1.11. Diethyl 3-ferrocenyl-1H-pyrrole-2,4-dicarboxylate (**14**)*

To a mixture of ethyl isocyanide (28 mmol, 2 equiv) and DBU (28 mmol, 2 equiv) dissolved in tetrahydrofuran (40 mL) was added dropwise ferrocene-carboxaldehyde (14 mmol, 1 equiv) in tetrahydrofuran (14 mL) at 45–50 °C for a period of 20 min with stirring. After stirring for 5 h at same temperature, the reaction mixture was neutralized with acetic acid and then the solvent was removed under reduced pressure. The resulting residue was extracted with ethyl acetate and the extract was washed with 10% HCl then with water, dried over Na_2SO_4. The solvents were evaporated, and the residue was purified by flash column chromatography using AcOEt/cyclohexane (30/70 − *v/v*) as eluent to afford **14**. Orange oil (37%); $R_f = 029$. IR (KBr) 3320 (NH), 1690 (C═O). ^1H NMR ($CDCl_3$) δ: 9.39 (s, 1H, NH), 7.40 (s, 1H, H-5), 5.17 (s, 2H, Cp-ortho), 4.48 (s, 2H, Cp-meta), 4.34–4.17 (m, 9H, Cp′ and CH_2), 1.44 (t, 3H, $J = 7.20$ Hz, CH_3), 1.37 (t, 3H, $J = 7.20$ Hz, CH_3). Anal. Calcd. for $C_{20}H_{21}NO_4Fe$: C, 60.78; H, 5.36; N, 3.54. Found: C, 60.90; H, 5.42; N, 3.52.

*6.1.12. Diethyl 3-ferrocenyl-1-(2-nitrophenyl)pyrrole-2,4-dicarboxylate (**15**)*

To a solution of diethyl 3-ferrocenylpyrrole-2,4-dicarboxylate **14** (2.53 mmol, 1 equiv) in 12 mL of DMF was added 1-fluoro-2-nitrobenzene (3.04 mmol, 1.2 equiv) and cesium carbonate (3.04 mmol, 1.2 equiv). The reaction mixture was refluxed for 1 h 30 min, then diluted in AcOEt (35 mL) after cooling. The reaction mixture was filtered, washed with water (2 × 35 mL), then brine (30 mL) and dried over sodium sulphate. The organic layer was concentrated under vacuo to give an orange oil which was purified by column chromatography using AcOEt/cyclohexane (20/80 − *v/v*) as eluent to yield **15**. Red crystals (54%); mp 120 °C $R_f = 025$. IR (KBr) 1695 (C═O). ^1H NMR ($CDCl_3$) δ: 8.13 (d, 1H, $J = 7.60$ Hz, H-3′), 7.80–7.61 (m, 2H, H-4′ and H-5′), 7.44 (m, 1H, H-6′), 7.37 (s, 1H, H-5), 5.07 (s, 2H, Cp-ortho), 4.47 (s, 2H, Cp-meta), 4.30–4.17 (m, 7H, Cp′ and CH_2), 4.03 (q, 2H, $J = 6.55$ Hz, CH_2), 1.36 (t, 3H, $J = 6.55$ Hz, CH_3), 1.01 (t, 3H, $J = 6.55$ Hz, CH_3). Anal. Calcd. for $C_{26}H_{24}N_2O_6Fe$: C, 60.48; H, 4.69; N, 5.43. Found: C, 60.64; H, 4.82; N, 5.37.

*6.1.13. Ethyl 4-oxo-3-ferrocenyl-5H-pyrrolo[1,2-a]quinoxaline-2-carboxylate (**16**)*

A suspension of **15** (2.7 mmol, 1 equiv) and iron powder (10.8 mmol, 4 equiv) in 12 mL of acetic acid was heated under reflux for 1 h 30 min. The reaction mixture was cooled, suspended in 30 mL of a 1 M aqueous solution of HCl, agitated, then filtered off, washed with HCl 1 M (10 mL), water, Et_2O and dried to give **16**. Orange crystals (66%); mp > 260 °C. IR (KBr) 3200–2810 (NH), 1710 and 1655 (C═O). ^1H NMR (DMSO-d_6) δ: 11.19 (s, 1H, NH), 8.65 (s, 1H, H-1), 8.19 (d, 1H, $J = 8.20$ Hz, H-9), 7.35–7.16 (m, 3H, H-6, H-7 and H-8), 5.00 (s, 2H, Cp-ortho), 4.34 (q, 2H, $J = 7.10$ Hz, CH_2), 4.29 (s, 2H, Cp-meta), 4.00 (s, 5H, Cp′), 1.36 (t, 3H, $J = 7.10$ Hz, CH_3). Anal.

Calcd. for $C_{24}H_{20}N_2O_3Fe$: C, 65.47; H, 4.58; N, 6.36. Found: C, 65.64; H, 4.52; N, 6.55.

6.1.14. Ethyl 4-chloro-3-ferrocenyl pyrrolo[1,2-a]quinoxaline-2-carboxylate (**17**)

A solution of 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one **6** (1.77 mmol) in $POCl_3$ (8 mL) was refluxed for 2 h. After removing excess of reactive under *vacuum*, the residue was carefully dissolved in water at 0 °C and the resulting solution was made basic with sodium carbonate. The precipitate was filtered, washed with water, dried and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate and evaporated to dryness. The crude residue was then purified by column chromatography using dichloromethane as eluent to give **6**. Red crystals (25%); mp 208 °C. $R_f = 0.56$. IR (KBr) 1715 (C═O). ^{1}H NMR ($CDCl_3$) δ: 8.28 (s, 1H, H-1), 7.89–7.85 (m, 2H, H-9 and H-6), 7.55–7.50 (m, 2H, H-7 and H-8), 5.24 (s, 2H, Cp-ortho), 4.63 (s, 2H, Cp-meta), 4.40–4.34 (m, 7H, Cp′ and CH_2), 1.48 (t, 3H, $J = 7.10$ Hz, CH_3). Anal. Calcd. for $C_{24}H_{19}ClN_2O_2Fe$: C, 62.84; H, 4.17; N, 6.11. Found: C, 62.97; H, 4.02; N, 6.31.

6.1.15. General procedure for 1-(3-ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl)piperazine (**2a**) and ethyl 3-ferrocenyl-4-piperazin-1-ylpyrrolo[1,2-a]quinoxaline-2-carboxylate, mono hydrate (**2b**)

A mixture of compound **7** or **13** (0.75 mmol, 1 equiv) and dry piperazine (7.5 mmol, 10 equiv) in ethylene glycol (7 mL) was heated at 140 °C for 2 h under nitrogen. After cooling the mixture was poured into crushed ice and extracted with chloroform. The organic layers were washed with brine, dried, and concentrated. The residue was chromatographed using CH_2Cl_2/methanol (90/10–*v*/*v*) as eluent to afford compound **2a** or **2b**.

6.1.15.1. 1-(3-Ferrocenyl-2-methylpyrrolo[1,2-a]quinoxalin-4-yl) piperazine (**2a**).

Orange crystals (91%); mp 123 °C. $R_f = 0.24$. IR (KBr) 3410 (NH). ^{1}H NMR ($CDCl_3$) δ: 7.69 (s, 1H, H-1′), 7.66 (d, 1H, $J = 7.85$ Hz, H-9′), 7.64 (d, 1H, $J = 7.85$ Hz, H-6′), 7.30–7.27 (m, 2H, H-7′ and H-8′), 4.65 (t, 2H, $J = 1.70$ Hz, Cp-ortho), 4.32 (t, 2H, $J = 1.70$ Hz, Cp-meta), 4.05 (s, 5H, Cp′), 3.34–3.30 (m, 2H, CH_2), 3.04–2.95 (m, 2H, CH_2), 2.78–2.72 (m, 4H, CH_2), 2.67 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 155.8 (C-4), 137.0 (C-5a), 128.9 (C-6), 127.7 (C-9a), 125.9 (C-7), 125.8 (C-8), 125.2 (C-3a), 119.1 (C-3), 118.9 (C-2), 115.7 (C-9), 114.2 (C-1), 83.0 (Cp-1), 71.8 (Cp-ortho), 70.8 (Cp′), 68.9 (Cp-meta), 51.5 (CH_2 pip.), 46.2 (CH_2 pip.), 14.0 (CH_3). Anal. Calcd. for $C_{26}H_{26}N_4Fe$: C, 69.34; H, 5.82; N, 12.44. Found: C, 69.51; H, 5.93; N, 12.48.

6.1.15.2. Ethyl 3-ferrocenyl-4-piperazin-1-ylpyrrolo[1,2-a]quinoxaline-2-carboxylate, mono hydrate (**2b**).

Orange crystals (46%); mp 150 °C. $R_f = 0.23$. IR (KBr) 3425 (NH), 1720 (C═O). ^{1}H NMR ($CDCl_3$) δ: 8.35 (s, 1H, H-1), 7.79 (dd, 1H, $J = 7.90$ and 1.50 Hz, H-9), 7.67 (dd, 1H, $J = 7.90$ and 1.50 Hz, H-6), 7.39 (ddd, 1H, $J = 7.90$, 7.20 and 1.50 Hz, H-8), 7.35 (ddd, 1H, $J = 7.90$, 7.20 and 1.50 Hz, H-7), 4.89 (s, 2H, Cp-ortho), 4.81 (bs, 2H, H_2O), 4.48 (q, 2H, $J = 7.20$ Hz, CH_2), 4.35 (s, 2H, Cp-meta), 4.02 (s, 5H, Cp′), 3.38–3.29 (m, 4H, CH_2), 2.85–2.77 (m, 4H, CH_2), 1.50 (t, 3H, $J = 7.20$ Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.5 (CO), 155.2 (C-4), 137.5 (C-5a), 129.3 (C-6), 127.5 (C-7), 127.1 (C-9a), 126.4 (C-8), 121.9 (C-3a), 121.7 (C-3), 120.3 (C-1), 120.2 (C-2), 114.7 (C-9), 80.9 (Cp-1), 74.2 (Cp-ortho), 71.0 (Cp′), 69.4 (Cp-meta), 62.1 (OCH_2), 49.5 (CH_2 pip.), 44.7 (CH_2 pip.), 16.0 (CH_3). Anal. Calcd. for $C_{28}H_{28}N_4O_2Fe.H_2O$: C, 63.89; H, 5.74; N, 10.64. Found: C, 63.98; H, 5.57; N, 10.76.

6.2. X-ray data

The structure of compounds **4** and **7** has been established by X-ray crystallography (Figs. 4 and 5). Orange single crystal ($0.10 \times 0.10 \times 0.02$ mm^3) of **4** was obtained by slow evaporation from methanol/chloroform (30/70) solution: triclinic, space group P-1, $a = 7.3059(2)$ Å, $b = 7.3059(2)$ Å, $c = 14.2791(5)$ Å, $\alpha = 94.216(2)°$, $\beta = 94.216(2)°$, $\gamma = 102.30°$, $V = 739.54(4)$ Å^3, $Z = 2$, δ (calcd) = 1.514 Mg m^{-3}, FW = 337.19 for $C_{18}H_{19}NO_2Fe$, $F(000) = 352$. Red single crystal ($0.15 \times 0.10 \times 0.05$ mm^3) of **7** was obtained by slow evaporation from methanol/chloroform (30/70) solution: Orthorhombic, space group P n a 21, $a = 16.326(3)$ Å, $b = 6.023(2)$ Å, $c = 17.993(3)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 1769.3(7)$ Å^3, $Z = 4$, δ (calcd) = 1.504 Mg m^{-3}, FW = 400.68 for $C_{22}H_{17}ClN_2Fe$, $F(000) = 824$. The measurements of the very thin crystals of **4** have been performed at the CRISTAL beamline of the SOLEIL synchrotron (France). A pseudo space group have been investigated with two parameters (a and b) and two angles (α and β) being equal. A cell reduction [51] of these triclinic parameters gave new parameters corresponding to a monoclinic C space group. The used transform matrix (1 1 0 −1 1 0 0 0 1) did not give identical Structure Factors between F(−h k l) and F(h k −l). This structure being centrosymmetric with 4 molecules in the unit cell, such a space group is not possible according to the conventional space groups. Intensities of **7** were collected with an Enraf-Nonius CAD-4 diffactometer using the CuKα radiation and a graphite monochromator up to $\theta = 55°$. Full crystallographic results have been deposited at the Cambridge Crystallographic Data Centre (CCDC-766981 and CCDC-766982), UK, as supplementary Material [52]. The data were corrected for Lorentz and polarization effects and for empirical absorption correction [53]. The structure was solved by direct methods Shelx 86 [54] and refined using Shelx 93 [55] suite of programs.

6.3. Biological assays

6.3.1. 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 [56]. *In vitro* antiplasmodial activity was determined using a modification of the semi-automated microdilution technique of Desjardins et al. [57] *P. falciparum* CQ-sensitive (F32/Tanzania) 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.

6.3.2. Inhibition of β-Hematin formation

Chloroquine (20 mM stock solution in water) was included in each series of experiments as positive control. Tested drugs **1d**, **1f-g**, **1i** and **1k-l**.were dissolved in MeOH/DMSO (4/1) and 20 mM stock solutions kept at 4 °C for a maximum of 10 days. The inhibition of β-hematin formation was tested using the technique described by Egan et al. [58] and modified by Baelmens et al. [59]. Briefly, 100 μL of a fresh 6.5 mM solution of hemin dissolved in 0.2 M NaOH was mixed with 200 μL of 3 M sodium acetate, 25 μL of 17.4 M acetic acid and 25 μL of the tested drug or relevant solvent as negative control. After a 24h-incubation at 37 °C under shaking, the supernatant

resulting from centrifugation for 15 min at 3300*g* was discarded and the pellet washed with 200 μL DMSO. This latter step was repeated once and, after a final wash with water, the pellet was dissolved in 200 μL 0.1 M NaOH. After a further 1/8 dilution, absorption at 405 nm was read using a Multiskan EX multiplate reader (Thermo Labsystems, Issy les Moulineaux, France). Results are expressed as percentage of inhibition of β-hematin formation as compared to the relevant negative control.

6.3.3. Cytotoxicity test upon human embryonic cells

A human diploid embryonic lung cell line (MRC-5, Bio-Whittaker 72211D) was used to assess the cytotoxic effects towards eukaryotic host cells. MRC-5 cells were seeded at 5000 cells *per* well in 100 μl. After 24 h, the cells were washed and two-fold dilutions of the drug were added in 200 μl standard culture medium (RPMI medium + 5% fetal calf serum) and maintained for five days under 5% CO_2 atmosphere. The final DMSO concentration in the culture remained below 0.1%. Untreated cultures were included as controls. The cytotoxicity was determined using the colorimetric MTT assay according to the manufacturer's recommendations (Cell proliferation kit I, Roche Applied Science, France) and scored as a percentage of reduction in absorption at 540 nm of treated cultures versus untreated control cultures. IC_{50} values were obtained from the drug concentration-response curve. The results were expressed as the mean ± the standard deviations determined from several independent experiments. The index of selectivity was defined as the ratio of the IC_{50} value on MRC-5 to that of *P. falciparum*.

6.4. Partition coefficients-Log D (pH 7.4 and 5.0)

In this study, the relative log *D* (pH 7.4 and 5.0) was assessed at pH 7.4 and 5.0 by the micro-HPLC method. Determinations were performed with a chromatographic apparatus (Spectra Series, San Jose, USA) equipped with a model P1000XR pump and a model SCM 1000 *vacuum* membrane degasser, a model UV 150 ultraviolet detector (λ = 220 nm) and a ChromJet data module integrator (ThermoFinnigan, San Jose, USA). The reversed phase column used, was a WatersXTerra^{TM}MS C_{18} (3.9 × 150 mm; 5 μm particle size) with a mobile phase consisting of acetonitrile − , acetonitrile − phosphate buffer (pH = 6) (60:40, *v/v* (**1c, 8, 9**)), acetonitrile − phosphate buffer (pH = 7) (60:40, *v/v* (**1b**, **1d-f**, **1h**, **1j-l**, **2a-b**)), (55:45, *v/v* (**1a**, **1g**, **1i**)), (50:50, *v/v* (**12**)), (40:60, *v/v* (**10**)). The compounds were partitioned between *n*-octanol (HPLC grade) and phosphate buffer (pH = 7.4). Octanol was presatured with the adequate phosphate buffer (1%), and conversely. 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 concentration of 50 μg/mL. Under the above-described chromatographic conditions, 20 μL of aqueous phase was injected into the chromatograph, leading to the determination of a peak area before partitioning (W_0). In screw-capped tubes, 1000 μL of the aqueous phase (V_{aq}) was then added to 100 μL of *n*-octanol (V_{oct}). The mixture was shaken by mechanical rotation during 30 min, followed by centrifugation achieved at 3000 rpm during 20 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). For each compound, the log *D* value was calculated using the formula: $\log D = \log [(W_0 - W_1)V_{aq}/W_1V_{oct}]$.

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References

[1] S.I. Hay, C.A. Guerra, A.J. Tatem, A.M. Noor, R.W. Snow, Lancet Infect. Dis. 4 (2004) 327–336.
[2] J.G. Breman, Am. J. Trop. Med. Hyg. 64 (2001) 1–11.
[3] S.M. Klan, A.P. Waters, Trends Parasitol. 20 (2004) 575–580.
[4] World Health Organization, 2010. http://www.who.int/drugresistance/en/.
[5] P.A. Winstanley, Parasitol. Today 16 (2000) 146–153.
[6] A.M. Oduola, W.K. Milhous, L.A. Salako, O. Walker, R.E. Desjardins, Lancet 2 (1987) 1304–1305.
[7] C. Biot, K. Chibale, Infect. Disord. Drug Targets 6 (2006) 173–204.
[8] A.L. Margaret, M.A.L. Blackie, P. Beagley, S.L. Croft, H. Kendrick, J.R. Moss, K. Chibale, Bioorg. Med. Chem. 15 (2007) 6510–6516.
[9] M.A. Blackie, K. Chibale, Met. Based Drugs (2008) 495123.
[10] N. Chavain, C. Biot, Biofutur 306 (2010) 50–53.
[11] X. Wu, M.L. Go, ^{26}Fe The use of iron-based drugs in medicine. in: M. Gielen, E.R.T. Tiekink (Eds.), Metallotherapeutic drugs and metal-based diagnostic agents. The use of metals in medicine, first ed. John Wiley & Sons Ltd., Chichester, England, 2005, pp. 179–200.
[12] C. Biot, G. Glorian, L.A. Maciejewski, J.S. Brocard, O. Domarle, G. Blampain, P. Millet, A.J. Georges, H. Abessolo, D. Dive, J. Lebibi, J. Med. Chem. 40 (1997) 3715–3718.
[13] D. Dive, C. Biot, Chem. Med. Chem. 3 (2008) 383–391.
[14] O. Domarle, G. Blampain, H. Agnaniet, T. Nzadiyabi, J. Lebibi, J.S. Brocard, L.A. Maciejewski, C. Biot, A.J. Georges, P. Millet, Antimicrob. Chemother. 42 (1998) 540–544.
[15] C. Attake, J. Mezui Me Ndong, A. Aubouy, L.A. Maciejewski, J. Brocard, J. Lébibi, P. Deloron, J. Antimicrob. Chemother. 51 (2003) 1021–1024.
[16] C. Biot, Curr. Med. Chem. – Anti-Infective Agents 3 (2004) 135–147.
[17] C. Biot, L. Delhaes, C.M. N'Diaye, L.A. Maciejewski, D. Camus, D. Dive, J.S. Brocard, Bioorg. Med. Chem. 7 (1999) 2843–2847.
[18] C. Biot, W. Daher, C.M. N'Diaye, P. Melnyk, B. Pradines, N. Chavain, A. Pellet, L. Fraisse, L. Pelinski, C. Jarry, J. Brocard, J. Khalife, I. Forfar-Bares, D. Dive, J. Med. Chem. 49 (2006) 4707–4714.
[19] S. Paitayatat, B. Tarnchompoo, Y. Thebtaranonth, Y. Yuthavong, J. Med. Chem. 40 (1997) 633–638.
[20] A. Baramee, A. Coppin, M. Mortuaire, L. Pelinski, S. Tomavo, J. Brocard, Bioorg. Med. Chem. 14 (2006) 1294–1302.
[21] F. Dubar, G. Anquetin, B. Pradines, D. Dive, J. Khalife, C. Biot, J. Med. Chem. 52 (2009) 7954–7957.
[22] J. Guillon, S. Moreau, E. Mouray, V. Sinou, I. Forfar, S. Belisle-Fabre, V. Desplat, P. Millet, D. Parzy, C. Jarry, P. Grellier, Bioorg. Med. Chem. 16 (2008) 9133–9144.
[23] J. Guillon, I. Forfar, M. Mamani-Matsuda, V. Desplat, M. Saliège, D. Thiolat, S. Massip, A. Tabourier, J.-M. Léger, B. Dufaure, G. Haumont, C. Jarry, D. Mossalayi, Bioorg. Med. Chem. 15 (2007) 194–210.
[24] J. Guillon, I. Forfar, V. Desplat, S. Belisle-Fabre, D. Thiolat, S. Massip, H. Carrie, D. Mossalayi, C. Jarry, J. Enzyme Inhib. Med. Chem. 22 (2007) 541–549.
[25] J. Guillon, P. Grellier, M. Labaied, P. Sonnet, J.-M. Léger, R. Déprez-Poulain, I. Forfar-Bares, P. Dallemagne, N. Lemaître, F. Péhourcq, J. Rochette, C. Sergheraert, C. Jarry, J. Med. Chem. 47 (2004) 1997–2009.
[26] V. Desplat, S. Moreau, A. Gay, S. Belisle-Fabre, D. Thiolat, S. Massip, D. Mossalayi, C. Jarry, J. Guillon, J. Enz. Inhib. Med. Chem. 25 (2010) 204–215.
[27] P.B. Madrid, A.P. Liou, J.L. DeRisi, R.K. Guy, J. Med. Chem. 49 (2006) 4535–4543.
[28] B. Kuhn, P. Mohr, M. Stahl, J. Med. Chem. 53 (2010) 2601–2611.
[29] J. Guillon, H. Dumoulin, P. Dallemagne, R. Reynolds, S. Rault, Pharm. Pharmacol. Commun. 4 (1998) 33–38.
[30] J.P. Alazard, O. Boyé, B. Gillet, D. Guénard, J.C. Beloeil, C. Thal, Bull. Soc. Chim. Fr. 130 (1993) 779–787.
[31] Y. Shimoji, K. Tomita, T. Hashimoto, F. Saito, Y. Morisawa, H. Mizuno, R. Yrikane, H. Koike, J. Med. Chem. 35 (1992) 816–822.
[32] D.H.R. Barton, J. Kervagoret, S.Z. Zard, Tetrahedron 46 (1990) 7587–7598.
[33] H. Dumoulin, S. Rault, M. Robba, J. Heterocyclic Chem. 34 (1997) 13–16.
[34] C.K. Chang, N. Bag, J. Org. Chem. 60 (1995) 7030–7032.
[35] L. Zhang, W. Meier, E. Wats, T.D. Costello, P. Ma, C.L. Ensinger, J.M. Rodgers, I.C. Jacobson, P. Rajagopalan, Tetrahedron Lett. 36 (1995) 8387–8390.
[36] M.J. Beach, R. Hope, H.K. Dieter, R.K. Rusell, Synth. Commun. 25 (1995) 2165–2183.
[37] Schann, S., Mayer, S., Gardan, S. Eur Patent 2007, 1798233 A1; [Chem. Abstr. 147 72808].
[38] A.P. Krapcho, C.S. Kuell, Synth. Commun. 20 (1990) 2559–2564.
[39] S. Fixon-Owoo, F. Levasseur, K. Williams, T.N. Sabado, M. Lowe, M. Klose, A.J. Mercier, P. Fields, J. Atkinson, Phytochemistry 63 (2003) 315–334.
[40] D. De, L.D. Byers, D.J. Krogstad, J. Heterocyclic Chem. 34 (1997) 315–320.
[41] A. Blum, J. Böttcher, B. Sammet, T. Luksch, A. Heine, G. Klebe, W.E. Diederich, Bioorg. Med. Chem. 16 (2008) 8574–8586.
[42] G. Campiani, E. Morelli, S. Gemma, V. Nacci, S. Butini, M. Hamon, C. Novellino, G. Greco, A. Cagnotto, M. Goegan, L. Cervo, F. Dalla Valle, C. Fracasso, S. Caccia, T. Mennini, J. Med. Chem. 42 (1999) 4362–4379.
[43] M. Suzuki, M. Miyoshi, K. Matsumoto, J. Org. Chem. 39 (1974) 1980.
[44] T.J. Egan, H.M. Marques, Coord. Chem. Rev. 190–192 (1999) 493–517.
[45] S.R. Hawley, P.G. Bray, M. Mungthin, J.D. Atkinson, P.M. O'Neill, S.A. Ward, Antimicrob. Agents Chemother. 42 (1998) 682–686.
[46] R. Buller, M.L. Peterson, Ö Almarsson, L. Leiserowitz, Cryst. Growth Des. 2 (2002) 553–562.

[47] D.J. Sullivan, Int. J. Parasitol. 32 (2002) 1645–1653.
[48] D.J. Sullivan, H. Matile, R.G. Ridley, D.E. Goldberg, J. Biol. Chem. 273 (1998) 31103–31107.
[49] A.F.G. Slater, A. Cerami, Nature 355 (1992) 167–169.
[50] S.K. Bhal, K. Kassam, G.I. Peirson, G.M. Pearl, Mol. Pharm. 4 (2007) 556–560.
[51] Y. Le Page, J. Appl. Cryst. 20 (1987) 264–269.
[52] Supplementary X-ray crystallographic data: Cambridge Crystallographic Data Centre, University Chemical Lab, Lensfield Road, Cambridge, CB2 1EW, UK; E-mail: deposit@chemcrys.cam.ac.uk.
[53] A.C.T. North, D.C. Phillips, F.S. Mathews, Acta Crystallogr. A24 (1968) 351.
[54] G.M. Sheldrick, C. Kröger, R. Goddard, SHELX 86 in Crystallographic Computing 3. Oxford University Press, New-York, 1985, 175.
[55] G.M. Sheldrick, SHELX 93, Program for the Refinement of the Crystal Structures. University of Göttingen, Germany, 1993.
[56] P. Grellier, A. Valentin, V. Millerioux, J. Schrével, D. Rigomier, Antimicrob. Agents Chemother. 38 (1994) 1144–1148.
[57] R.E. Desjardins, C.J. Canfield, J.D. Haynes, J.D. Chulay, Antimicrob. Agents Chemother. 16 (1979) 710–718.
[58] T.J. Egan, D.C. Ross, P.A. Adams, FEBS. Lett. 352 (1994) 54–57.
[59] R. Baelmans, E. Deharo, V. Munõz, M. Sauvain, H. Ginsburg, H. Exp, Parasitol 96 (2000) 243–248.