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Antimalarial activities of ferroquine conjugates with either glutathione reductase inhibitors or glutathione depletors via a hydrolyzable amide linker

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ABSTRACT

Based on the prodrug concept as well as the combination of two different classes of antimalarial agents, we designed and synthesized two series of ferrocenic antimalarial dual molecules consisting of a ferroquine analogue conjugated with a glutathione reductase inhibitor (or a glutathione depletor) through a cleavable amide bond in order to target two essential pathways in the malarial parasites. The results showed no enhancement of the antimalarial activity of the dual molecules but evidenced a unique mode of action of ferroquine and ferrocenyl analogues distinct of those of chloroquine and nonferrocenic 4-aminoquinoline analogues.

1. Introduction

Malaria is one of the most common infectious diseases and an enormous public-health problem. It is an important cause of death and illness in tropical and subtropical countries. It causes 600 million clinical cases and one to three million deaths each year, mostly among young children. Mortality has risen in recent years, probably due to the increase of resistance to antimalarial treatments.[1] Chloroquine (CQ, Fig. 1) was the most widely used antimalarial drug. Unfortunately, resistance to CQ has spread all over the malaria endemic areas[2,3] and resistance to sulphadoxine–pyrimethamine[4,5] has followed rapidly. Drug-resistant strains of malaria parasites offer strong challenges for drug discovery progress.

As an alternative to CQ, the discovery of ferroquine (FQ, SR97193) has been a significant achievement (Fig. 1).[6–8] FQ has an excellent antimalarial activity profile, both in vitro and in vivo, especially with potent antiplasmodial effects against CQ-resistant parasites.[9–12] FQ is currently being developed by Sanofi-Aventis and has entered into phase II clinical trials in autumn 2007 in combination with artesunate (ARS) as recommended by WHO.[13,14]

Recent investigations aimed at understanding how the ferrocene core in FQ contributes toward a stronger antiplasmodial activity than CQ, were undertaken to probe the real contribution of redox properties of the ferrocene (Fe^{2+})/ferricinium (Fe^{3+}) system as a possible discriminating property.[15] Besides a higher lipophilicity that influences its pharmacodynamic behavior, FQ was shown to be able to generate low amounts of hydroxyl radicals from H_2O_2 via a Fenton-like reaction upon specific oxidizing conditions found in the parasitic digestive vacuole (DV).

It was also observed that an elevation of the glutathione (GSH) content in *Plasmodium falciparum* leads to an increased resistance to CQ, while GSH depletion in resistant strains restores sensitivity to CQ.[16] Indeed, GSH is known to protect the parasite from oxidative damage and recently, GSH was showed to be involved in the Fenton-based degradation of toxic heme by continuously recycling the PFIX(Fe^{3+}) into PFIX(Fe^{2+}) in the malaria parasite.[17] GSH is found almost exclusively in its reduced form and high intracellular GSH levels depend on the efficient reduction of glutathione disulfide (GSSG) by glutathione disulfide reductase (GR). Thus, GR inhibitors were developed to fight malaria[18] and to reverse the CQ-resistance.[19,20]

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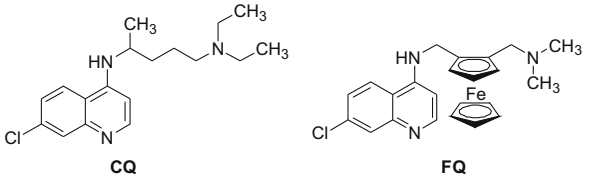


Figure 1. Chemical structures of chloroquine (CQ) and ferroquine (FQ).

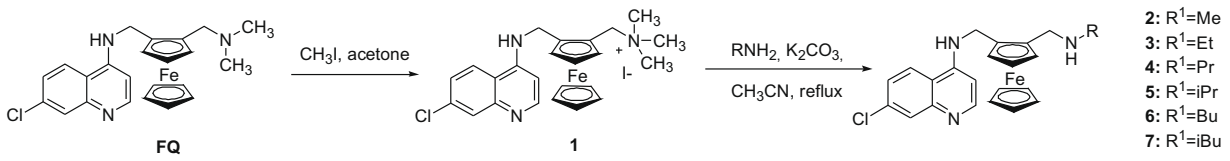
To avoid the spread of resistance, Peters and co-workers were the first to show that drug combination strategy could be beneficial to the treatment of malaria,[21] and that a judicious combination of antimalarial drugs could delay the selection of resistant mutants in vitro.[22–24] As simple drug combinations can cause problems due to different pharmacokinetics, the binding of active molecules via a covalent linker seems to be a good alternative. Indeed, the binding of both active entities allows to increase the bioavailability of the final dual molecule and to merge active molecules with independent modes of action to prevent the emergence of resistance.[25] This dual prodrug strategy has been applied to malaria[20,26–34] and to various other diseases such as tuberculosis,[35] cancer,[36] and HIV.[37–39] In particular, combination of two active components targeting the heme detoxification pathway and the thiol network of malaria parasites was shown to provide the proof of concept both in vitro and in vivo, and at the molecular level.[20] The nature of the covalent linker plays an essential role since the cleavage needs to occur under the specific conditions found in the target cells, for example, the heme detoxification pathway in the malarial parasites, and ideally not before the dual molecule reaches the pathogen.

Based on this new strategy, we used the FQ moiety as a template for the design of new antimalarial ferrocenic dual molecules. These organometallic compounds combine the core portions of two structurally distinct moieties via an appropriate linker. As side chain modification of FQ did not greatly affect its antimalarial activity,[40] a FQ derivative was linked to a GR inhibitor[20,41] or GSH depletor,[42] previously demonstrated to lower the GR activity or the GSH content in the cells, respectively. Herein, we report the synthesis and in vitro antiparasitic activity on *P. falciparum* susceptible (NF54) and resistant (K1) strains of two new series of organometallic dual molecules. Special attention was paid to the characterization of the products and to the investigation of their mechanism(s) of action. Preliminary cleavage assays were conducted to support the prodrug strategy. Despite a lower antimalarial activity of the new final molecules compared to FQ itself, our studies evidenced a unique mode of action of FQ and ferrocenyl analogues compared to CQ and related nonferrocenic 4-aminoquinolines.

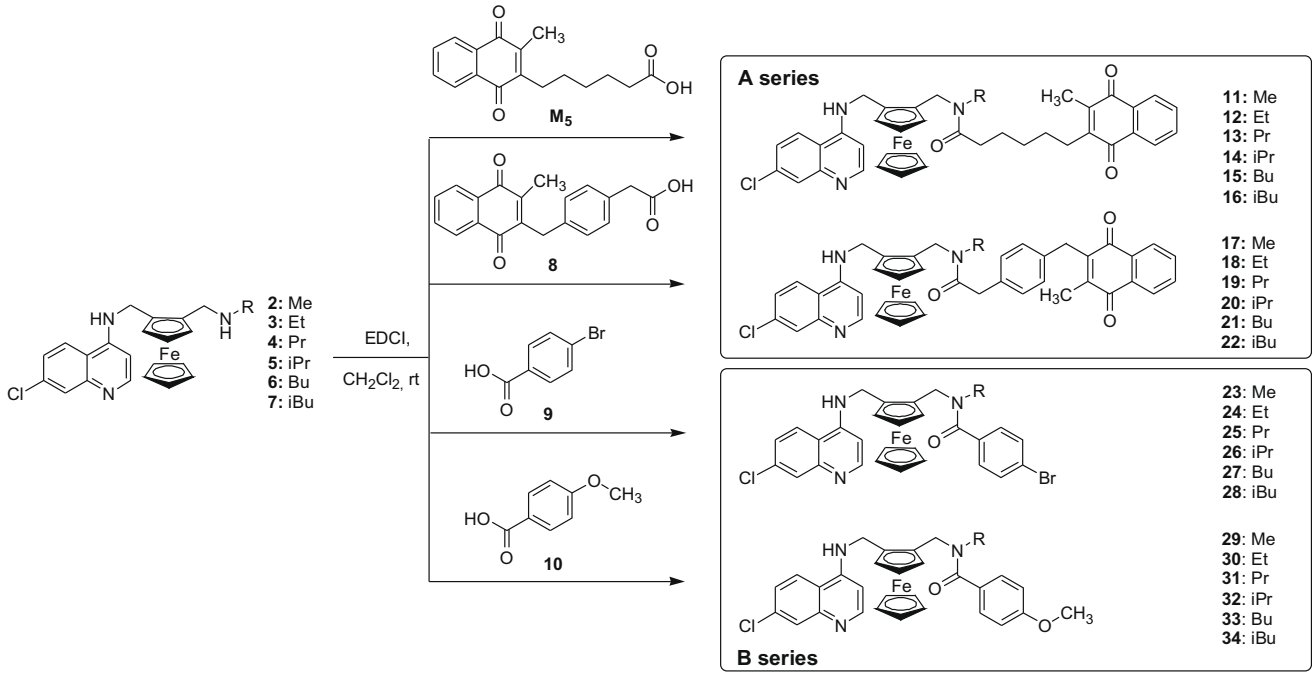
2. Results

2.1. Chemistry

The secondary amine FQ analogues **2–7** (Scheme 1), were prepared from FQ by nucleophilic substitution.[40] The basic terminal nitrogen atom of FQ was quaternized with methyl iodide in acetone at room temperature, and the resulting quaternary ammonium was then substituted by the appropriate amine to afford



Scheme 1. Synthesis of FQ amino derivatives **2–7**.



Scheme 2. Synthesis of the dual molecules **11–34**.

the products **2–7** in moderate (24%) to good (74%) yields. The dual molecules were readily prepared by coupling the corresponding amine with the different carboxylic acids in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) as the coupling reagent (Scheme 2). Starting **M_5** and **8** involved in the A series were synthesized as previously described[41,43] while both *p*-substituted benzoic acids **9** and **10** used in B series were commercially available. After completion of the coupling reaction, the desired products **11–34** were obtained in moderate (23%) to good (77%) yields.

2.2. In vitro antimalarial activity

The antimalarial activity of the secondary amine FQ analogues **2–7** and the new dual molecules **11–34** was assessed against both CQ-susceptible (NF54) and CQ-resistant (K1) clones of *P. falciparum* (Tables 1 and 2). CQ, FQ, and ARS were used as controls for the study.

All the secondary amines **2–7** displayed IC_{50} values in the low nanomolar range, similar to that of FQ and in the same order as for ARS. Noteworthy is to mention that four new FQ analogues **4–7** displayed more potent antimalarial effects against the CQ-resistant K1 strain than FQ itself, even if no broad difference in antimalarial activities among these derivatives was observed. As previously noted, a slight modification of the basic side chain does not affect the activity if the substituents are not too large.[40]

In the first group of A series, the potency of the dual molecules **11–16** against both CQ-susceptible and CQ-resistant clones was highly increased when compared to the GR inhibitor **M_5** alone (IC_{50} = 43.3 μM on the CQ-resistant *P. falciparum* strain K1[41]) but decreased when compared to the corresponding amines **2–7**, and also to FQ (Table 1). Nevertheless, these compounds, in particular dual molecules **11–13**, showed high antimalarial activity (with IC_{50} <100 nM, below the threshold of CQ-resistance) on both laboratory strains. They appeared far more efficient than CQ against the K1 strain (IC_{50}, 3–7-fold lower). In the second group of A series, the dual molecules **17–22**, as the dual molecules **11–16**, were more potent than the GR inhibitor **8** (IC_{50} = 6.2 μM on the CQ-resistant *P. falciparum* strain K1[41]) but their antimalarial activities were decreased compared to the FQ analogues **2–7**. Worse, these new compounds **17–22** were not able to counter the CQ-resistance, except compound **18** whose IC_{50} value is 35.8 nM against K1.

In B series, the dual molecules **23–34**, including the GSH depletors **9** and **10**, showed potent antimalarial activities in the low nanomolar range (Table 2). They were more active than CQ against the resistant strain but less active than FQ. With compounds **29–30** the dose–response curves could not reach the 100% growth inhibition. As previously observed with the A series, compounds of the B series were also less active than the corresponding amines **2–7**.

Table 1
In vitro sensitivities of *P. falciparum* strains toward the FQ amine derivatives **2–7** and the dual molecules **11–22** of A series

Compd	Type	GR inhibitor-based	NF54 IC_{50} (nM)	K1 IC_{50} (nM)
2	FQ analogue	—	7.5	15.6
3	FQ analogue	—	13.0	11.2
4	FQ analogue	—	9.4	7.0
5	FQ analogue	—	8.0	7.2
6	FQ analogue	—	14.9	5.1
7	FQ analogue	—	12.0	8.0
11	Dual molecule	M_5	48.7	36.6
12	Dual molecule	M_5	44.2	27.4
13	Dual molecule	M_5	92.2	26.7
14	Dual molecule	M_5	42.6	40.8
15	Dual molecule	M_5	104.2	58.8
16	Dual molecule	M_5	51.4	67.8
17	Dual molecule	8	205.8	186.3
18	Dual molecule	8	90.4	35.8
19	Dual molecule	8	318.9	252.9
20	Dual molecule	8	384.3	306.8
21	Dual molecule	8	336.2	333.8
22	Dual molecule	8	389.0	444.8
CQ	Reference	—	5.1	206.7
ARS	Reference	—	9.8	3.4
FQ	Reference	—	9.3	11.0

Table 2
In vitro sensitivities of *P. falciparum* strains toward the dual molecules **23–34** of B series

Compd	Type	GSH depletor-based	NF54 IC_{50} (nM)	K1 IC_{50} (nM)
23	Dual molecule	9	50.8	53.2
24	Dual molecule	9	80.6	79.2
25	Dual molecule	9	44.0	57.8
26	Dual molecule	9	71.7	105.7
27	Dual molecule	9	62.9	68.0
28	Dual molecule	9	127.4	161.2
29	Dual molecule	10	61.5	93.4
30	Dual molecule	10	68.3	114.8
31	Dual molecule	10	44.0	55.9
32	Dual molecule	10	48.0	67.7
33	Dual molecule	10	58.1	80.2
34	Dual molecule	10	77.6	151.6
CQ	Reference	—	11.9	289.2
ARS	Reference	—	5.1	2.9
FQ	Reference	—	11.6	16.1

2.3. Glutathione reductase inhibition studies

The dual molecules and also FQ were tested for their ability whether or not to inhibit the *P. falciparum* GR activity. The GR inhibitor **M_5** was taken as a reference compound for the test. As previously reported, an IC_{50} value of 4.5 μM was determined for **M_5** in the GR-catalyzed 1 mM GSSG reduction assay in the presence of 100 μM of NADPH.[41,44]

As expected, no inhibition was observed for FQ. The IC_{50} value was higher than 100 μM. In accordance with the prodrug approach, dual molecules showed no inhibition of the *P. falciparum* GR. Only a slight inhibition was observed for the dual molecule **13** with an IC_{50} value of 25 μM in the 1 mM GSSG reduction assay.

2.4. β-Hematin inhibition assay

The most active compounds on *P. falciparum* strains were tested for their ability to inhibit the formation of β-hematin, the synthetic

equivalent of hemozoin.[45,46] The compounds were incubated at 60 °C during 1 h in the presence of hematin at pH 4.5. After incubation, the reaction mixture is quenched with a pyridine solution. If the compound inhibits the formation of β-hematin, free hematin is released and forms a complex with pyridine, which absorbs at 405 nm. If the compound does not inhibit the formation of β-hematin, no absorption is observed at 405 nm. The original version of Egan's assay[47] was slightly modified as recently reported.[32,48] The IC_{50} values were defined as the number of drug equivalents, related to 1 equiv of hematin, necessary for reaching 50% of maximal inhibition. They were determined at increasing concentrations of the drug from 0 to 5 mM.

Figure 2 shows the pyridine:hematin complex formation evidencing inhibition of β-hematin formation as a function of increasing drug:hematin ratio in the presence of amodiaquine (AQ), FQ and the dual molecule **18**. All curves displayed were recorded in the presence of DMSO selected as the solvent to solubilize the drug. At zero equivalent of drug, water bound to hematin likely prevents the binding of pyridine resulting in a higher absorption at 405 nm, and then is displaced at increasing drug concentration. As reported in Tables 3 and 4, FQ analogues **2–7** showed a high inhibition of β-hematin formation with little variation in the IC_{50} values close to that of FQ, except compound **2**. The dual molecules incorporating the GR inhibitors (compounds **13** and **18**) or the GSH depletors (compounds **25** and **31**) also showed a high inhibition in the same range as FQ, and even higher by taking into account the very low drug:hematin ratio (0.25:1 for **13** and **25**, and 0.5:1 for **31** vs 0.75:1 for FQ), necessary to reach the maximal inhibition. This evidences that a low level of these dual molecules is enough to displace the solvent from hematin and to interact with it with high affinity. Interestingly, these two compounds are also the most potent antimalarial compounds within their respective series, and possess the same alkylation pattern (R = Pr) at the terminal amide group of the side chain. Other short FQ analogues also showed a lower drug:hematin ratio compared to FQ (0.5:1 for **3**, **6**, and **7** vs 0.75:1 for FQ); they also possess a more potent antimalarial activity than FQ itself. Both FQ analogues **4** and **5**, which behave like FQ in the β-hematin test, that is, with the same drug:hematin ratio (0.75:1), but with a higher maximal% inhibition at this ratio (84% and 90%, respectively, vs 76% for FQ), are indeed more potent antimalarials against both CQ-susceptible and CQ-resistant *P. falciparum* strains. Hence, based on these data it seems that FQ appears to combine the ideal and best data set of results (β-hematin inhibition, affinity to hematin, catalyst for Fenton reaction) to express the highest antimalarial activity. But, other short FQ analogues designed here, like **4** and **5** (and to a lesser degree **6** and **7**), are also very close to FQ and even better.

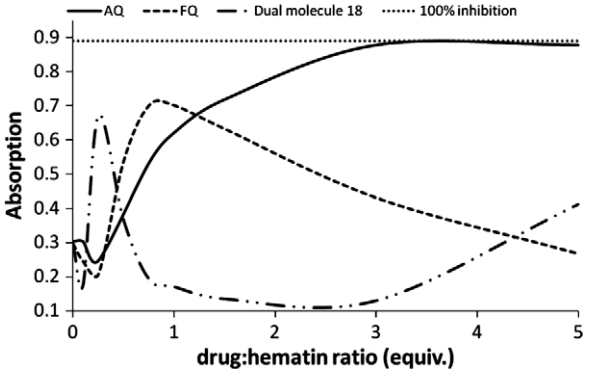


Figure 2. Examples of β-hematin inhibition curves in DMSO.

Table 3
In vitro inhibition of β-hematin with secondary amine derivatives **2–7** in MeOH

Compd	Type	Max. inhibition reached (%) (at drug:hematin ratio)	IC_{50}
2	FQ analogue	83 (1.5:1)	0.6
3	FQ analogue	81 (0.5:1)	0.2
4	FQ analogue	84 (0.75:1)	0.3
5	FQ analogue	90 (0.75:1)	0.2
6	FQ analogue	58 (0.5:1)	0.2
7	FQ analogue	76 (0.5:1)	0.2
CQ	Reference	71 (2:1)	1.3
AQ	Reference	100 (1.5:1)	0.7
FQ	Reference	76 (0.75:1)	0.3

Table 4
In vitro inhibition of β-hematin with selected dual molecules (**13**, **18**, **25** and **31**) in DMSO

Compd	Type	Max. inhibition reached (%) (at drug:hematin ratio)	IC_{50}
13	A series	79 (0.25:1)	0.2
18	A series	87 (0.75:1)	0.5
25	B series	86 (0.25:1)	0.2
31	B series	79 (0.5:1)	0.2
CQ	Reference	89 (3:1)	1.9
AQ	Reference	100 (1.5:1)	0.7
FQ	Reference	86 (0.75:1)	0.4

As previously discussed, all ferrocenic compounds described herein show a different inhibition profile than AQ (Fig. 2).[49] Whereas AQ had a sigmoid behavior like CQ, our compounds (FQ itself, secondary amines **2–7** and dual molecules **13**, **18**, **25**, and **31**) had a maximum and showed a decreased inhibition or non-inhibition at high concentration like FQ (bell-shaped curve). Noteworthy is that rare cases of bell-shaped curves in the β-hematin assay observed at increasing concentration of drug:hematin ratio, were also observed: (i) for a dual molecule built from a short CQ analogue and the GR inhibitor $\mathbf{M_5}$, via a tertiary amide bond,[32] and (ii) for halofantrine (T. J. Egan, personal communication). Halofantrine was recently shown to coordinate to the Fe^{3+} center through its alcohol functionality—via an alkoxide Fe^{3+}–O bond—in addition to π-stacking of the phenanthrene ring over the porphyrin of bound hematin.[49]

2.5. Cleavage of dual molecules under biomimetic conditions

The stability of the dual molecules toward chemical hydrolysis under the specific conditions (acidic and oxidizing) of the parasitic DV and under the conditions mimicking the cytosol was evaluated and compared. All experiments were carried out at a temperature of 37 °C. For the cytosolic conditions, the pH was maintained at 7.4 whereas for the vacuolar conditions a pH of 5.2 was fixed and the experiments were carried out in the presence of hematin and H_2O_2 to mimic the oxidative conditions. The supernatant of each reaction mixture was analyzed by HPLC and the retention times (HPLC t_R) of starting materials and products were determined.

At cytosolic pH (pH 7.4), without hematin and without H_2O_2, all the dual molecules were stable with half-lives longer than 20 h, and no cleavage of the amide bond was observed.

At vacuolar pH (pH 5.2) the reaction of 5 mM dual molecules **13** (HPLC t_R = 20.10 min), **25** (HPLC t_R = 20.19 min), or **31** (HPLC t_R = 17.50 min) in the presence of 1 mM hematin, and 15 mM hydrogen peroxide, resulted in the total disappearance of the starting dual molecule accompanied by the release of the GR inhibitor ($\mathbf{M_5}$, HPLC t_R = 18.14 min; or compound **8**, HPLC t_R = 18.11 min) or the GSH depletor (compound **10**, HPLC t_R = 15.12 min), respectively. The half-lives of the compounds were estimated between

0.5 and 1 h. Surprisingly, no matter which alkyl group was considered, the second peak (HPLC t_R = 12.43 min) did not correspond to the secondary amines **2–7**. First attempts to isolate (by preparative HPLC) and to characterize (by NMR and MS) this second metabolic counterpart failed. Due to the similarity of the retention times between the 4-aminoquinoline biometabolites regardless of the choice of the alkyl substituent, the similar experiment was performed with FQ. In the specific conditions of the DV of the parasite (pH 5.2, hematin, H_2O_2, 37 °C), the complete disappearance of FQ (HPLC t_R = 13.20 min) was accompanied with the formation of a new product after 1 h reaction as evidenced by the peak at 12.43 min. NMR and MS experiments were carried out to identify the compound (HPLC t_R = 12.43 min) formed in the metabolic study of FQ. In the DV conditions, FQ evolved toward the formation of 4-amino-7-chloroquinoline (confirmed by MS (*m/z* 177) and ^{13}C-HSQC in D_20 at 280 K and 400 MHz, see Section 4).

2.6. Intramolecular hydrogen bond

Recently, the importance of the intramolecular hydrogen bond in neutral FQ for its antimalarial activity was probed.[48] From these experiments, it could be concluded that the extended structure of diprotonated FQ in water changes in neutral FQ to a more compact conformation via an additional intramolecular hydrogen bond in apolar media. This flip/flop hydrogen bond between the open conformation (charged FQ) and the folded conformation (uncharged FQ) was supposed to help transport from water to the hydrophobic membranes. The presence of an intramolecular hydrogen bond in the new A and B series was also investigated here. Whereas we were not able to obtain crystals from A series, we succeeded in B series.

Slow recrystallization from dichloromethane produced orange crystals of **29** which were suitable for X-ray crystallography (Table SI1). An intramolecular hydrogen bond appears prominent in the crystal structure of dual molecule **29** (Fig. SI2), with a O27···N11 distance of 2.90 Å. As a consequence, only the *s*-cis conformer of **29** is observed in the solid state. This nine-membered pseudocycle forces the ferrocenyl moiety to be almost orthogonal to the quinoline plane, with the torsion angle C4–N11–C12–C13 being −76.7°. As a result, the distances between the two diastereotopic protons from C12 and C23 are very different. The distance between the protons H12b (pro-*R*) and H23b (pro-*S*) is 3.53 Å while the distance between the protons H12a (pro-*S*) and H23a (pro-*R*) is 2.12 Å. No special arrangement is noted in the packing diagram.

In order to confirm whether the conformational structure observed in the crystals was similar in apolar solvent, NMR experiments were carried out with compound **29** in $CDCl_3$. Spatial proximities were assessed through a NOESY experiment. The occurrence of a NOE between the protons H25 of the methyl group on the amido N24 and the aromatic protons H29–H33 (Table SI3) and the absence of NOE between methylenic protons H23 with aromatic protons H29–H33 show that the amide bond adopts a *s*-cis configuration in solution, as observed in the solid structure. The particular pattern of NOEs (H12a–H23a, H23b–H25, H12b–H23b) confirms the eclipsed conformation of the ferrocene. An additional NOE between the protons H5 and H29–H33 can also prove the proximity of the phenyl group and the quinoline ring and supposes the presence of an intramolecular hydrogen bond. A large downfield shift (2 ppm) for H23b proton is observed due to the anisotropic deshielding effect of the carbonyl from the amide group.

3. Discussion

Our strategy is aimed at the design of antimalarial dual molecules based on a FQ analogue attached to a GR inhibitor or a GSH depletor via an amide linker, which could be cleaved by hydrolysis or enzymatically into the parasite.[20,32,50] Our previous studies with FQ analogues **2–7** confirmed that a slight modification of the terminal nitrogen atom (numbered N24) did not affect the antimalarial activity and neither the ability to inhibit the β-hematin formation as previously reported (Tables 1 and 3).[40] So, the substituents (Me, Et, Pr, *i*Pr, Bu, and *i*Bu) were chosen in order to keep the most efficient antimalarial activity of FQ. The selection of the GR inhibitors (the carboxylic acids $\mathbf{M_5}$ and **8**) was based on data from Davioud–Charvet's group.[20,41] For GSH depletors, 4-bromobenzoic acid and 4-methoxybenzoic acid were used as masked phenol, which are known to react with thiols following oxidative metabolism.[42,51]

Among the two series, the dual molecules based on a FQ analogue attached to $\mathbf{M_5}$ (first group of A series, compounds **11–16**) were found to be the most active in vitro against *P. falciparum* strains, with IC_{50} values in the nanomolar range (Table 1). Regardless of the choice of the alkyl group on the nitrogen atom N24, the dual molecules **11–16** were more active on both strains than the GR inhibitor $\mathbf{M_5}$. Nevertheless, their antimalarial activity was slightly decreased compared to the parent FQ analogues **2–7** alone. This decrease in the antimalarial activity of the dual molecules compared with that of FQ analogues might be explained by the fact that both the amide bond and the side chain of the FQ derivative are cleaved following the oxidative metabolism in the DV.

Preliminary tests on the mechanism of action were carried out. We have shown that some compounds are inhibitors of β-hematin formation as potent as FQ and analogues (Table 4). Moreover, they have the same inhibition profile as FQ and its analogues. They have a maximum and show a decreased inhibition or non-inhibition at high concentration as previously discussed for FQ (bell-shaped curve).[48] It was also shown that the new dual molecules inhibit the GR enzyme to a lower extent than $\mathbf{M_5}$ itself. As our dual molecules can be considered as prodrugs, this result was expected because the carboxylic function of the naphthoquinone moiety is required for the affinity to the target GR enzyme (establishment of a salt bridge with an Arg residue).[41]

A characterization study (X-ray crystallization and NMR experiments) was also carried out. Like FQ, the dual molecules are able to establish an intramolecular hydrogen bond, which gives a specific conformation to the molecules. This may help the dual molecules to cross the membranes and consequently, enter the DV, site of hemozoin crystallization. On the other hand, the amide bond also suppresses the basicity of the terminal tertiary amine which was previously reported as being important for the antimalarial activity.[32,52,53] Indeed, this terminal amine is supposed to be essential for an effective accumulation of the drug into the DV of CQ-susceptible parasites, but not for a potent antimalarial activity against CQ-resistant parasites.[32,52,53] Thus, for DV accumulation of the drugs containing no basic tertiary amine, CQ-resistant parasites might express modified drug transport mechanisms, either at the uptake or the efflux levels. Nevertheless, it seems not to alter in a significant way the antimalarial activity. These results are in agreement with the results obtained in a nonferrocenic series.[32,52,53]

FQ was recently reported to catalyze the Fenton reaction under the specific oxidizing conditions of the parasitic DV.[15] While this local production of reactive oxygen species appeared not sufficient to affect the stability of FQ, it has been proposed to be sufficient to yield significant damage to FQ-enriched membranes of the parasitic DV. But, this production of hydroxyl radicals could also allow the destruction of hematin itself, in agreement with the apparent slow decrease of inhibition of β-hematin formation observed in the presence of FQ. This earlier observation was first interpreted as a possible phenomenon of self-aggregation of the ferrocenyl drug to explain the apparent lower drug concentration in the assay.[48] In the view of the present data with all dual molecules built

through a tertiary amide bond from a short FQ analogue and a GR inhibitor, all displaying a sharp bell-shaped curve of inhibition of β-hematin formation as illustrated with compound **18** (Fig. 2), it seems that the apparent decrease of inhibition of β-hematin formation at increasing concentration of drug:hematin ratio, could be interpreted as the result of a lower concentration of hematin in the assay or to a lower concentration of the drug following degradation by Fenton reaction. Consequently, the lower antimalarial activity of the very potent β-hematin inhibitors, like **13**, **18**, **25**, and **31** versus FQ, might result from high Fenton catalyst properties, and destruction of the hematin or the drug itself at too high a rate. Competition within the DV between hemozoin formation (detoxification in the absence of the drug), and oxidative damage of the parasite components upon concentration of free heme in the membranes might be affected by drugs that can too quickly destroy the drug:hematin ratio by Fenton reaction, and finally prevent the oxidative damage. Ideally, FQ seems to have the capability to combine high lipophilicity, high affinity to hematin to form complexes with hematin and to inhibit its conversion into hemozoin during the heme detoxification pathway, and appropriate redox behavior to favor the leak of hematin in the membranes and to prevent a too high rate of destruction of hematin:drug ratio by Fenton reaction within the DV. This work emphasizes the high potential of FQ and short analogues as antimalarial drug candidates according to a redox mechanism distinct from the mode of action of CQ and analogues.

4. Experimental section

4.1. Chemistry

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded at room temperature on a Bruker AC 300 spectrometer. TMS was used as an internal standard and $CDCl_3$ as the solvent. ^{1}H NMR analyses were obtained at 300 MHz (s: singlet, d: doublet, t: triplet, dd: double doublet, m: multiplet); whereas ^{13}C NMR analyses were obtained at 75.4 MHz. The chemical shifts (δ) are given in parts per million relative to TMS (δ = 0.00). Mass spectra were recorded by means of a Waters Micromass Quattro II triple quadrupole LC mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources. Melting points were determined on a Köfler apparatus and are uncorrected. Column chromatography, carried out on silica gel (Merck Kieselgel 60) was used for the purification of compounds. Reactions were monitored by thin-layer chromatography (TLC) using coated silica gel plates, detection by UV lamp. The HRMS and elemental analyses were carried out at the Service Central d'Analyse from the CNRS in Solaize. The purity (P_{HPLC}) of the compounds was checked by two types of high pressure liquid chromatography (HPLC) columns, a Macherey-Nagel C18 Nucleosil column (4 × 300 mm, 5 μm, 100 Å) or Macherey-Nagel EC 250/4.6 Nucleodur 100-5 CN-RP (4 × 300 mm, 5 μm, 100 Å). Analytical HPLC was performed on a Spectra system equipped with a UV detector set at 254 nm. Compounds were dissolved in acetonitrile and injected through a 50 μL loop. The following solvent systems were used: eluent (A): 0.05% trifluoroacetic acid (TFA) in H_2O, eluent (B) 100% CH_3CN. HPLC retention times (HPLC t_R) were determined at a flow rate of 1 mL/min, using the following conditions: 100% eluent A for 5 min, then a gradient run to 100% eluent B over the next 20 min.

4.2. General procedure for preparation of the secondary and tertiary amine FQ derivatives 2–7

A mixture of the corresponding amine (methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl-, diethyl-, dipropyl-, diisopropyl, and dibutylamine, 10 mmol) and *N*-(7-chloro-4-quinolyl)-*N*-2-[(1,1,1-trimethylammonio)methyl] ferrocenylmethylamine iodide recently prepared (1 mmol) was dissolved in acetonitrile (25 mL). Potassium carbonate (10 mmol) was added in excess, and the mixture was stirred for 3–7 h at reflux. After cooling to room temperature, the reaction was quenched with water (25 mL). The aqueous layer was then extracted with dichloromethane (3 × 50 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc, 10% triethylamine).

4.2.1. 7-Chloro-*N*-(3-((methylamino)methyl)ferrocenyl) quinolin-4-amine (2)

Following the general procedure, a yellow solid was obtained: yield 74%; mp 185 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.52 (d, 1H, *J* = 5.4 Hz), 7.91 (d, 1H, *J* = 2.1 Hz), 7.79 (d, 1H, *J* = 9.0 Hz), 7.68 (m, 1H), 7.27 (dd, 1H, *J* = 2.2; 9.0 Hz), 6.45 (d, 1H, *J* = 5.4 Hz), 4.34 (d, 1H, *J* = 13.1 Hz), 4.26 (m, 1H), 4.19 (m, 1H), 4.15 (m, 1H), 4.14 (s, 5H), 4.09 (m, 1H), 3.68 (d, 1H, *J* = 12.3 Hz), 3.47 (d, 1H, *J* = 12.3 Hz), 2.44 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 151.0 (CH), 149.3 (C^{IV}), 148.2 (C^{IV}), 133.5 (C^{IV}), 127.3 (CH), 123.7 (CH), 121.6 (CH), 116.8 (C^{IV}), 97.8 (CH), 84.1 (C^{IV}), 82.5 (C^{IV}), 69.5 (CH), 69.3 (CH), 68.1 (5CH), 65.0 (CH), 48.9 (CH_2), 41.4 (CH_2), 35.2 (CH_3); MS *m/z* 422 MH^+ ^{37}Cl, 421 $M^{+\cdot}$ ^{37}Cl, 420 MH^+ ^{35}Cl, 419 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37}Cl−$NHCH_3)^+$, 389 (M ^{35}Cl−$NHCH_3)^+$; Anal Calcd for $C_{22}H_{22}N_3ClFe$: C, 62.95; H, 5.28; N, 10.01; Found: C, 63.03; H, 5.52; N, 10.25; HPLC t_R C18 Nucleosil 14.4 min, CN-RP 13.3 min.

4.2.2. 7-Chloro-*N*-(3-((ethylamino)methyl)ferrocenyl)quinolin-4-amine (3)

A yellow solid was obtained: yield 26%; mp 207 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, *J* = 5.4 Hz), 7.92 (d, 1H, *J* = 2.1 Hz), 7.83 (d, 1H, *J* = 9.0 Hz), 7.35 (m, 1H), 7.25 (dd, 1H, *J* = 2.1; 9.1 Hz), 6.47 (d, 1H, *J* = 5.4 Hz), 4.36 (d, 1H, *J* = 13.1 Hz), 4.26 (m, 1H), 4.20 (m, 1H), 4.15 (m, 1H), 4.14 (s, 5H), 4.11 (m, 1H), 4.09 (s, 1H), 3.70 (d, 1H, *J* = 12.2 Hz), 3.52 (d, 1H, *J* = 12.2 Hz), 2.70 (m, 2H), 1.13 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 150.8 (CH), 148.8 (C^{IV}), 148.0 (C^{IV}), 133.3 (C^{IV}), 127.1 (CH), 123.4 (CH), 121.6 (CH), 116.6 (C^{IV}), 97.7 (CH), 84.3 (C^{IV}), 82.2 (C^{IV}), 69.2 (CH), 69.0 (CH), 67.9 (5CH), 64.8 (CH), 46.6 (CH_2), 42.8 (CH_2), 41.1 (CH_2), 13.8 (CH_3); MS *m/z* 436 MH^+ ^{37}Cl, 435 $M^{+\cdot}$ ^{37}Cl, 434 MH^+ ^{35}Cl, 433 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37}Cl−$NHCH_2CH_3)^+$, 389 (M ^{35} Cl−$NHCH_2CH_3)^+$, 179 ($NH_3C_8H_5N^{37}Cl)^+$; Anal Calcd for $C_{23}H_{24}N_3ClFe$: C, 63.69; H, 5.58; N, 9.69; Found: C, 63.35; H, 5.83; N, 9.37; HPLC t_R C18 Nucleosil 14.5 min, CN-RP 13.5 min.

4.2.3. 7-Chloro-*N*-(3-((propylamino)methyl)ferrocenyl) quinolin-4-amine (4)

A yellow solid was obtained: yield 40%; 135 δ 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.51 (d, 1H, *J* = 5.4 Hz), 7.90 (d, 1H, *J* = 1.7 Hz), 7.84 (d, 1H, *J* = 9.0 Hz), 7.35 (m, 1H), 7.24 (dd, 1H, *J* = 2.1; 9.0 Hz), 6.46 (d, 1H, *J* = 5.4 Hz), 4.38 (d, 1H, *J* = 13.4 Hz), 4.28 (m, 1H), 4.22 (m, 1H), 4.17 (m, 1H), 4.15 (s, 5H), 4.10 (m, 1H), 3.75 (d, 1H, *J* = 12.1 Hz), 3.55 (d, 1H, *J* = 12.1 Hz), 2.64 (m, 2H), 1.56 (m, 2H), 0.90 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 150.9 (CH), 149.1 (C^{IV}), 148.1 (C^{IV}), 133.6 (C^{IV}), 127.2 (CH), 123.6 (CH), 121.8 (CH), 116.7 (C^{IV}), 97.8 (CH), 84.0 (C^{IV}), 82.5 (C^{IV}), 69.5 (CH), 69.3 (CH), 68.2 (5CH), 65.2 (CH), 50.6 (CH_2), 46.7 (CH_2), 41.2 (CH_2), 21.6 (CH_2), 10.7 (CH_3); MS *m/z* 450 MH^+ ^{37}Cl, 449 $M^{+\cdot}$ ^{37}Cl, 448 MH^+ ^{35}Cl, 447 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37}Cl−$NHCH_2CH_3)^+$, 389 (M ^{35}Cl−$NHCH_2CH_3)^+$, 179 ($NH_3C_8H_5N^{37}Cl)^+$; HPLC t_R C18 Nucleosil 14.7 min, CN-RP 13.8 min.

4.2.4. 7-Chloro-*N*-(3-((isopropylamino)methyl)ferrocenyl) quinolin-4-amine (5)

A yellow solid was obtained: yield 35%; mp 151 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 1H, *J* = 5.4 Hz), 7.91 (d, 1H, *J* = 1.0 Hz), 7.85 (d, 1H, *J* = 8.9 Hz), 7.25 (dd, 1H, *J* = 2.2; 8.9 Hz), 7.16 ppm (m, 1H), 6.48 (d,

1H, J = 5.4 Hz), 4.37 (d, 1H, J = 12.9 Hz), 4.25 (m, 1H), 4.20 (m, 1H), 4.16 (s, 5H), 4.14 (m, 1H), 4.09 (m, 1H), 3.71 (d, 1H, J = 12.0 Hz), 3.49 (d, 1H, J = 12.0 Hz), 2.89 (m, 1H), 1.11 (d, 3H, J = 6.3 Hz), 0.99 (d, 3H, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 152.2 (CH), 150.0 (C^{IV}), 149.3 (C^{IV}), 134.7 (C^{IV}), 128.4 (CH), 124.6 (CH), 123.1 (CH), 117.7 (C^{IV}), 99.0 (CH), 85.8 (C^{IV}), 83.5 (C^{IV}), 70.3 (CH), 70.2 (CH), 69.2 (5CH), 66.2 (CH), 49.6 (CH), 45.8 (CH_2), 42.2 (CH_2), 23.6 (CH_3), 22.0 (CH_3); MS m/z 450 MH^+ ^{37}Cl, 449 $M^{+\cdot}$ ^{37}Cl, 448 MH^+ ^{35}Cl, 447 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37} Cl−$NHCH_2CH_3)^+$, 389 (M ^{35} Cl−NHC $H_2CH_3)^+$, 179 $(NH_3C_8H_5N^{37}Cl)^+$; HPLC t_R C18 Nucleosil 14.6 min, CN-RP 13.8 min.

4.2.5 7-Chloro-*N*-(3-((butylamino)methyl)ferrocenyl)quinolin-4-amine (6)

A yellow solid was obtained: yield 41%; mp 132 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J = 5.4 Hz), 7.91 (d, 1H, J = 2.0 Hz), 7.80 (d, 1H, J = 9.0 Hz), 7.26 (m, 1H), 7.23 (dd, 1H, J = 2.2; 9.0 Hz), 6.47 (d, 1H, J = 5.4 Hz), 4.37 (d, 1H, J = 12.3 Hz), 4.26 (m, 1H), 4.19 (m, 1H), 4.15 (s, 5H), 4.14 (m, 1H), 4.08 (m, 1H), 3.71 (d, 1H, J = 11.8 Hz), 3.52 (d, 1H, J = 11.8 Hz), 2.64 (m, 2H), 1.50 (m, 2H), 1.31 (m, 2H), 0.90 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 151.1 (CH), 149.2 (C^{IV}), 148.2 (C^{IV}), 133.6 (C^{IV}), 127.3 (CH), 123.5 (CH), 121.9 (CH), 116.7 (C^{IV}), 97.9 (CH), 84.3 (C^{IV}), 82.5 (C^{IV}), 69.5 (CH), 69.2 (CH), 68.1 (5CH), 65.1 (CH), 48.5 (CH_2), 46.8 (CH_2), 41.3 (CH_2), 30.8 (CH_2), 19.5 (CH_2), 13.0 (CH_3); MS m/z 464 MH^+ ^{37}Cl, 463 $M^{+\cdot}$ ^{37}Cl, 462 MH^+ ^{35}Cl, 461 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37} Cl−$NHCH_2CH_3)^+$, 389 (M ^{35} Cl−$NHCH_2CH_3)^+$, 179 $(NH_3C_8H_5N^{37}Cl)^+$; HPLC t_R C18 Nucleosil 14.9 min, CN-RP 14.0 min.

4.2.67-Chloro-*N*-(3-((isobutylamino)methyl)ferrocenyl) quinolin-4-amine (7)

A yellow solid was obtained: yield 36%; mp 114 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J = 5.3 Hz), 7.91 (s, 1H), 7.76 (d, 1H, J = 8.9 Hz), 7.22 (d, 1H, J = 8.7 Hz), 7.12 (m, 1H), 6.47 (d, 1H, J = 5.3 Hz), 4.36 (d, 1H, J = 13.0 Hz), 4.26 (m, 1H), 4.20 (m, 1H), 4.15 (m, 1H), 4.14 (s, 5H), 4.09 (s, 1H), 3.70 (d, 1H, J = 12.6 Hz), 3.50 (d, 1H, J = 12.6 Hz), 2.46 (m, 2H), 1.84 (m, 1H), 0.90 (d, 6H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 151.1 (CH), 149.0 (C^{IV}), 148.3 (C^{IV}), 133.6 (C^{IV}), 127.4 (CH), 123.6 (CH), 121.6 (CH), 116.7 (C^{IV}), 97.9 (CH), 84.6 (C^{IV}), 82.5 (C^{IV}), 69.6 (CH), 69.2 (CH), 68.2 (5CH), 65.2 (CH), 56.8 (CH_2), 46.9 (CH_2), 41.2 (CH_2), 26.7 (CH), 19.6 (CH_3), 19.5 (CH_3); MS m/z 464 MH^+ ^{37}Cl, 463 $M^{+\cdot}$ ^{37}Cl, 462 MH^+ ^{35}Cl, 461 $M^{+\cdot}$ ^{35}Cl, 391 (M ^{37}Cl−$NHCH_2CH_3)^+$, 389 (M ^{35}Cl−$NHCH_2CH_3)^+$; HPLC t_R C18 Nucleosil 14.9 min, CN-RP 14.2 min.

4.3. General procedure for preparation of the dual drugs 11–34

A mixture of the corresponding amine (**2–7**, 1 mmol) and the corresponding carboxylic acid (**M_5**, **8**, **9**, or **10**) (1 mmol) was dissolved in dry dichloromethane (10 mL). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) (1 mmol) was added and the mixture was stirred for 3–7 h at room temperature. Then, the reaction was diluted with dichloromethane (20 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ (2 × 15 mL) and then with brine (15 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc, 2.5% triethylamine).

4.3.1. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-methyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (11)

Following the general procedure, a brown solid was obtained: yield 42%; mp 56 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J = 5.6 Hz), 8.07 (m, 2H), 7.89 (d, 1H, J = 9.1 Hz), 7.86 (d, 1H, J = 2.1 Hz), 7.69 (dd, 2H, J = 3.4; 5.8 Hz), 7.25 (dd, 1H, J = 2.1; 8.9 Hz), 6.96 (m, 1H), 6.53 (d, 1H, J = 5.6 Hz), 5.35 (d, 1H, J = 14.3 Hz), 4.50 (dd, 1H, J = 4.5; 14.7 Hz), 4.35 (m, 1H), 4.30 (m, 1H), 4.29 (m, 1H), 4.17 (s, 5H), 4.14 (t, 1H, J = 2.6 Hz), 3.59 (d, 1H, J = 14.3 Hz), 2.98 (s, 3H), 2.62 (t, 2H, J = 7.5 Hz), 2.31 (m, 2H), 2.18 (s, 3H), 1.67 (m, 2H), 1.48 (m, 2H), 1.43 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 184.3 (C^{IV}), 183.7 (C^{IV}), 172.1 (C^{IV}), 149.8 (CH), 149.1 (C^{IV}), 147.3 (C^{IV}), 146.0 (C^{IV}), 142.3 (C^{IV}), 134.1 (C^{IV}), 132.4 (2CH), 131.1 ($2C^{IV}$), 126.4 (CH), 125.3 (CH), 125.2 (CH), 123.9 (CH), 121.7 (CH), 116.2 (C^{IV}), 97.2 (CH), 82.6 (C^{IV}), 81.2 (C^{IV}), 69.5 (CH), 68.9 (CH), 68.3 (5CH), 66.7 (CH), 44.6 (CH_2), 39.4 (CH_2), 34.6 (CH_3), 32.5 (CH_2), 28.6 (CH_2), 27.5 (CH_2), 25.9 (CH_2), 23.7 (CH_2), 11.8 (CH_3); MS m/z 690 MH^+ ^{37}Cl, 689 $M^{+\cdot}$ ^{37}Cl, 688 MH^+ ^{35}Cl, 687 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}Cl$ $NHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 20.3 min, CN-RP 17.0 min.

4.3.2. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-ethyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (12)

A brown solid was obtained: yield 50%; mp 84 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 5.4 Hz), 8.07 (m, 2H), 7.88 (d, 1H, J = 9.0 Hz), 7.86 (d, 1H, J = 2.1 Hz), 7.68 (dd, 2H, J = 3.4; 5.7 Hz), 7.21 (dd, 1H, J = 2.2; 8.9 Hz), 6.93 (m, 1H), 6.52 (d, 1H, J = 5.4 Hz), 5.20 (d, 1H, J = 14.3 Hz), 4.49 (dd, 1H, J = 4.4; 14.3 Hz), 4.34 (m, 1H), 4.28 (m, 1H), 4.24 (m, 1H), 4.17 (s, 5H), 4.13 (m, 1H), 3.70 (d, 1H, J = 14.3 Hz), 3.31 (m, 2H), 2.62 (t, 2H, J = 7.4 Hz), 2.27 (m, 2H), 2.18 (s, 3H), 1.68 (m, 2H), 1.47 (m, 2H), 1.38 (m, 2H),1.16 (t, 3H, J = 7.01 Hz); ^{13}C NMR ($CDCl_3$) δ 185.6 (C^{IV}), 185.0 (C^{IV}), 173.0 (C^{IV}), 150.9 (CH), 150.5 (C^{IV}), 148.3 (C^{IV}), 147.3 (C^{IV}), 143.6 (C^{IV}), 135.5 (C^{IV}), 133.6 (2CH), 132.3 ($2C^{IV}$), 126.5 (CH), 125.3 (CH), 125.2 (CH), 122.9 (CH), 122.1 (CH), 117.5 (C^{IV}), 98.5 (CH), 83.7 (C^{IV}), 83.1 (C^{IV}), 70.7 (CH), 70.2 (CH), 69.5 (5CH), 67.8 (CH), 42.8 (CH_2), 42.2 (CH_2), 40.9 (CH_2), 33.1 (CH_2), 29.8 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 25.1 (CH_2), 13.9 (CH_3), 12.9 (CH_3); MS m/z 704 MH^+ ^{37}Cl, 703 $M^{+\cdot}$ ^{37}Cl, 702 MH^+ ^{35}Cl, 701 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}$ $ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 20.6 min, CN-RP 16.9 min.

4.3.3. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-propyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (13)

A brown solid was obtained: yield 27%; mp: decomposed before melting; ^{1}H NMR ($CDCl_3$) δ 8.50 (d, 1H, J = 4.9 Hz), 8.06 (m, 2H), 7.92 (d, 1H, J = 8.8 Hz), 7.89 (d, 1H, J = 2.1 Hz), 7.68 (dd, 2H, J = 3.3; 5.8 Hz), 7.22 (dd, 1H, J = 2.0; 9.0 Hz), 7.01 (m, 1H), 6.53 (d, 1H, J = 6.9 Hz), 5.19 (d, 1H, J = 14.7 Hz), 4.50 (dd, 1H, J = 4.9; 14.7 Hz), 4.33 (m, 1H), 4.29 (m, 1H), 4.24 (m, 1H), 4.18 (s, 5H), 4.14 (t, 1H, J = 2.5 Hz), 3.72 (d, 1H, J = 14.7 Hz), 3.20 (m, 2H), 2.62 (m, 2H), 2.30 (m, 2H), 2.17 (s, 3H), 1.67 (m, 2H), 1.58 (m, 2H), 1.49 (m, 2H), 1.27 (m, 2H), 0.92 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 185.3 (C^{IV}), 184.7 (C^{IV}), 172.9 (C^{IV}), 151.1 (CH), 150.1 (C^{IV}), 148.6 (C^{IV}), 147.0 (C^{IV}), 143.3 (C^{IV}), 135.0 (C^{IV}), 133.3 (2CH), 132.1 ($2C^{IV}$), 127.6 (CH), 126.3 (CH), 126.2 (CH), 124.8 (CH), 122.6 (CH), 117.3 (C^{IV}), 98.3 (CH), 83.6 (C^{IV}), 82.9 (C^{IV}), 70.4 (CH), 70.0 (CH), 69.3 (5CH), 67.5 (CH), 49.1 (CH_2), 43.0 (CH_2), 40.7 (CH_2), 32.9 (CH_2), 29.6 (CH_2), 28.5 (CH_2), 26.9 (CH_2), 25.0 (CH_2), 21.7 (CH_2), 12.7 (CH_3), 11.3 (CH_3); MS m/z 718 MH^+ ^{37}Cl, 717 $M^{+\cdot}$ ^{37}Cl, 716 MH^+ ^{35}Cl, 715 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 21.3 min, CN-RP 17.0 min; HRMS m/z calcd for $C_{41}H_{43}ClFeN_3O_3$ $(M+H)^+$ 716.2342, found 716.2347.

4.3.4. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-isopropyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (14)

A brown solid was obtained: yield 23%; mp: decomposed before melting; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, 1H, J = 5.6 Hz), 8.11 (d, 1H, J = 10.3 Hz), 7.99 (m, 2H), 7.86 (t, 1H, J = 2.6 Hz), 7.61 (dd, 2H, J = 3.9; 5.7 Hz), 7.19 (dd, 1H, J = 2.1; 8.9 Hz), 6.46 (d, 1H, J = 5.8 Hz), 4.38 (d, 1H, J = 14.4 Hz), 4.19 (m, 2H), 4.16 (m, 2H), 4.10 (s, 5H), 4.05 (m, 1H), 3.96 (d, 1H, J = 14.4 Hz), 2.55 (m, 2H), 2.21 (m, 2H), 2.09 (s, 3H), 1.60 (m, 2H), 1.39 (m, 2H), 1.31 (d, 1H, J = 6.8 Hz), 1.16

(dd, 6H, J = 4.4; 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 185.4 (C^{IV}), 184.8 (C^{IV}), 172.3 (C^{IV}), 151.8 (CH), 150.3 (C^{IV}), 149.2 (C^{IV}), 147.3 (C^{IV}), 143.4 (C^{IV}), 134.8 (C^{IV}), 133.5 (2CH), 132.2 ($2C^{IV}$), 128.0 (CH), 126.3 (CH), 126.2 (CH), 124.8 (CH), 123.4 (CH), 117.7 (C^{IV}), 98.5 (CH), 85.1 (C^{IV}), 82.7 (C^{IV}), 69.9 (CH), 69.7 (CH), 69.4 (5CH), 67.4 (CH), 48.5 (CH), 41.6 (CH_2), 39.1 (CH_2), 33.7 (CH_2), 29.6 (CH_2), 28.6 (CH_2), 27.0 (CH_2), 25.6 (CH_2), 22.6 (CH_3), 21.5 (CH_3), 12.9 (CH_3); MS m/z 718 MH^+ ^{37}Cl, 717 $M^{+\cdot}$ ^{37}Cl, 716 MH^+ ^{35}Cl, 715 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClN\ HCH_2\ C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 22.4 min, CN-RP 17.1 min.

4.3.5. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-butyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (15)

A brown solid was obtained: yield 29%; mp: decomposed before melting; ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J = 5.9 Hz), 8.06 (m, 2H), 7.90 (d, 1H, J = 9.2 Hz), 7.87 (d, 1H, J = 2.1 Hz), 7.68 (dd, 1H, J = 3.4; 5.7 Hz), 7.25 (dd, 1H, J = 2.0; 9.0 Hz), 6.98 (m, 1H), 6.53 (d, 1H, J = 5.9 Hz), 5.20 (d, 1H, J = 14.3 Hz), 4.49 (dd, 1H, J = 4.5; 14.4 Hz), 4.34 (m, 1H), 4.28 (m, 1H), 4.23 (m, 1H), 4.17 (s, 5H), 4.13 (m, 1H), 3.70 (d, 1H, J = 14.3 Hz), 3.21 (m, 2H), 2.61 (t, 2H, J = 6.4 Hz), 2.25 (m, 2H), 2.18 (s, 3H), 1.67 (m, 2H), 1.55 (m, 2H), 1.46 (m, 2H), 1.43 (m, 2H), 1.32 (m, 2H), 0.93 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 185.4 (C^{IV}), 184.8 (C^{IV}), 172.3 (C^{IV}), 151.8 (CH), 150.3 (C^{IV}), 149.2 (C^{IV}), 147.3 (C^{IV}), 143.4 (C^{IV}), 134.8 (C^{IV}), 133.5 (2CH), 132.2 ($2C^{IV}$), 128.0 (CH), 126.3 (CH), 126.2 (CH), 124.8 (CH), 123.4 (CH), 117.7 (C^{IV}), 98.5 (CH), 85.1 (C^{IV}), 82.7 (C^{IV}), 69.9 (CH), 69.7 (CH), 69.4 (5CH), 67.4 (CH), 48.5 (CH), 41.6 (CH_2), 39.1 (CH_2), 33.7 (CH_2), 29.6 (CH_2), 28.6 (CH_2), 27.0 (CH_2), 25.6 (CH_2), 22.6 (CH_3), 21.5 (CH_3), 12.9 (CH_3); MS m/z 732 MH^+ ^{37}Cl, 731 $M^{+\cdot}$ ^{37}Cl, 730 MH^+ ^{35}Cl, 729 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 22.2 min, CN-RP 17.5 min.

4.3.6. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocényl}-*N*-isobutyl-6-(3-methyl-1,4-naphtoquinolyl)hexanamide (16)

A brown solid was obtained: yield 32%; mp: decomposed before melting; ^{1}H NMR ($CDCl_3$) 8.49 (d, 1H, J = 5.3 Hz), 8.06 (m, 2H), 7.91 (d, 1H, J = 9.0 Hz), 7.87 (d, 1H, J = 2.0 Hz), 7.68 (dd, 2H, J = 3.4; 5.6 Hz), 7.25 (dd, 1H, J = 2.2; 9.0 Hz), 6.92 (m, 1H), 6.52 (d, 1H, J = 5.3 Hz), 5.17 (d, 1H, J = 14.4 Hz), 4.46 (dd, 1H, J = 4.2; 15.0 Hz), 4.32 (m, 1H), 4.28 (m, 1H), 4.22 (m, 1H), 4.17 (s, 5H), 4.13 (m, 1H), 3.76 (d, 1H, J = 14.4 Hz), 3.08 (m, 2H), 2.59 (t, 2H, J = 7.4 Hz), 2.24 (m, 2H), 2.16 (s, 3H), 2.00 (m, 1H), 1.65 (m, 2H), 1.43 (m, 2H), 1.31 (m, 2H), 0.90 (d, 3H, J = 6.5 Hz), 0.84 (d, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 184.3 (C^{IV}), 183.6 (C^{IV}), 172.1 (C^{IV}), 150.7 (CH), 148.8 (C^{IV}), 148.1 (C^{IV}), 146.0 (C^{IV}), 142.3 (C^{IV}), 133.7 (C^{IV}), 132.3 (2CH), 131.1 ($2C^{IV}$), 127.1 (CH), 125.2 (CH), 125.1 (CH), 123.7 (CH), 121.5 (CH), 116.5 (C^{IV}), 97.4 (CH), 82.7 (C^{IV}), 81.8 (C^{IV}), 69.3 (CH), 68.9 (CH), 68.2 (5CH), 66.5 (CH), 53.2 (CH_2), 52.4 (CH_2), 41.8 (CH_2), 39.8 (CH_2), 32.1 (CH_2), 28.6 (CH_2), 27.5 (CH_2), 26.2 (CH), 25.9 (CH_2), 23.9 (CH_2), 19.3 (CH_3), 18.8 (CH_3), 11.7 (CH_3); MS m/z 732 MH^+ ^{37}Cl, 731 $M^{+\cdot}$ ^{37}Cl, 730 MH^+ ^{35}Cl, 729 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 22.9 min, CN-RP 17.5 min.

4.3.7. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-methyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl] phenyl}acetamide (17)

A brown solid was obtained: yield 50%; mp 103 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 5.3 Hz), 8.07 (m, 2H), 7.84 (d, 1H, J = 1.8 Hz), 7.68 (m, 2H), 7.66 (d, 1H, J = 6.1 Hz), 7.15 (d, 2H, J = 8.0 Hz), 7.05 (d, 2H, J = 8.0 Hz), 7.04 (m, 1H), 6.91 (m, 1H), 6.51 (d, 1H, J = 5.3 Hz), 5.33 (d, 1H, J = 14.2 Hz), 4.47 (dd, 1H, J = 4.9; 14.6 Hz), 4.36 (m, 1H), 4.29 (m, 1H), 4.25 (m, 1H), 4.15 (s, 5H), 4.14 (m, 1H), 4.00 (s, 2H), 3.63 (dd, 2H, J = 4.8; 15.6 Hz), 3.53 (d, 1H, J = 15.3 Hz), 2.98 (s, 3H), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 184.3 (C^{IV}), 183.6 (C^{IV}), 170.3 (C^{IV}), 150.4 (CH), 148.7 (C^{IV}), 148.0 (C^{IV}), 144.1 (C^{IV}), 143.4 (C^{IV}), 135.8 (C^{IV}), 133.6 (C^{IV}), 132.5 (2CH), 131.5 (C^{IV}), 131.1 ($2C^{IV}$), 128.2 (2CH), 127.9 (2CH), 126.8 (CH), 125.4 (CH), 125.2 (CH), 123.8 (CH), 121.4 (CH), 116.3 (C^{IV}), 97.2 (CH), 82.9 (C^{IV}), 81.0 (C^{IV}), 69.5 (CH), 68.8 (CH), 68.2 (5CH), 66.7 (CH), 44.9 (CH_2), 39.5 (CH_2), 39.4 (CH_2), 35.0 (CH_3), 31.1 (CH_2), 12.3 (CH_3); MS m/z 724 MH^+ ^{37}Cl, 723 $M^{+\cdot}$ ^{37}Cl, 722 MH^+ ^{35}Cl, 721 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.8 min, CN-RP 16.9 min.

4.3.8. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-ethyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl]phenyl} acetamide (18)

A brown solid was obtained: yield 67%; mp 86 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (d, 1H, J = 5.4 Hz), 7.99 (m, 2H), 7.75 (d, 1H, J = 2.1 Hz), 7.59 (m, 2H), 7.00 (dd, 1H, J = 8.0; 19.9 Hz), 6.78 (m, 1H), 6.41 (d, 1H, J = 5.6 Hz), 5.08 (d, 1H, J = 14.2 Hz), 4.38 (dd, 1H, J = 4.3; 14.1 Hz), 4.27 (m, 1H), 4.16 (m, 2H), 4.07 m (s, 5H), 4.05 (m, 2H), 3.90 (s, 2H), 3.66 (d, 1H, J = 14.2 Hz), 3.50 (m, 2H), 3.27 (m, 2H), 2.13 (s, 3H), 1.17 (m, 2H), 1.01 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 184.2 (C^{IV}), 183.5 (C^{IV}), 169.9 (C^{IV}), 149.7 (CH), 149.1 (C^{IV}), 147.1 (C^{IV}), 144.0 (C^{IV}), 143.3 (C^{IV}), 135.7 (C^{IV}), 133.8 (C^{IV}), 132.4 (2CH), 131.9 (C^{IV}), 130.9 (C^{IV}), 130.8 (C^{IV}), 128.2 (2CH), 127.8 (2CH), 126.1 (CH), 125.3 (CH), 125.2 (CH), 123.9 (CH), 121.6 (CH), 116.2 (C^{IV}), 97.2 (CH), 82.5 (C^{IV}), 81.6 (C^{IV}), 69.3 (CH), 68.9 (CH), 68.3 (5CH), 66.6 (CH), 41.8 (CH_2), 41.5 (CH_2), 39.6 (CH_2), 38.8 (CH_2), 31.0 (CH_2), 12.7 (CH_3), 12.3 (CH_3); MS m/z 738 MH^+ ^{37}Cl, 737 $M^{+\cdot}$ ^{37}Cl, 736 MH^+ ^{35}Cl, 735 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 20.2 min, CN-RP 17.0 min.

4.3.9. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-propyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl] phenyl}-acetamide (19)

A brown solid was obtained: yield 64%; mp: decomposed before melting; ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 4.8 Hz), 8.06 (m, 2H), 7.85 (s, 1H), 7.67 (m, 2H), 7.64 (m, 1H), 7.11 (d, 2H, J = 7.2 Hz), 7.06 (m, 1H), 7.02 (d, 2H, J = 7.1 Hz), 6.90 (m, 1H), 6.50 (d, 1H, J = 5.3 Hz), 5.15 (d, 1H, J = 14.4 Hz), 4.45 (dd, 1H, J = 3.8; 14.3 Hz), 4.34 (m, 1H), 4.28 (m, 1H), 4.23 (m, 1H), 4.16 (s, 5H), 4.14 (m, 1H), 3.97 (s, 2H), 3.76 (d, 1H, J = 14.4 Hz), 3.58 (q, 2H, J = 14.7 Hz), 3.22 (m, 2H), 2.22 (s, 3H), 1.52 (m, 2H), 0.85 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 185.5 (C^{IV}), 184.7 (C^{IV}), 171.2 (C^{IV}), 151.3 (CH), 150.0 (C^{IV}), 148.8 (C^{IV}), 145.3 (C^{IV}), 144.5 (C^{IV}), 136.9 (C^{IV}), 134.9 (C^{IV}), 133.6 (2CH), 133.1 (C^{IV}), 132.2 (C^{IV}), 132.1 (C^{IV}), 129.2 (CH), 129.0 (CH), 127.8 (2CH), 126.5 (CH), 126.4 (CH), 125.0 (CH), 122.5 (CH), 119.1 (CH), 117.4 (C^{IV}), 98.4 (CH), 83.8 (C^{IV}), 82.8 (C^{IV}), 70.5 (CH), 70.0 (CH), 69.4 (5CH), 67.8 (CH), 49.7 (CH_2), 43.2 (CH_2), 40.8 (CH_2), 40.2 (CH_2), 32.2 (CH_2), 21.9 (CH_2), 13.4 (CH_3), 11.3 (CH_3); MS m/z 752 MH^+ ^{37}Cl, 751 $M^{+\cdot}$ ^{37}Cl, 750 MH^+ ^{35}Cl, 749 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 21.2 min, CN-RP 17.1 min; HRMS m/z calcd for $C_{44}H_{41}ClFeN_3O_3$ $(M+H)^+$ 750.2186, found 750.2172.

4.3.10. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-isopropyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl] phenyl}acetamide (20)

A brown solid was obtained: yield 55%; mp 105 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 5.2 Hz), 8.05 (m, 2H), 7.83 (m, 1H), 7.67 (m, 2H), 7.64 (m, 1H), 7.11 (d, 2H, J = 7.9 Hz), 7.07 (m, 1H), 7.02 (d, 2H, J = 6.8 Hz), 6.84 (m, 1H), 6.49 (d, 1H, J = 5.2 Hz), 5.15 (d, 1H, J = 14.6 Hz), 4.43 (dd, 1H, J = 3.8; 14.4 Hz), 4.34 (m, 1H), 4.27 (m, 1H), 4.22 (m, 1H), 4.15 (s, 5H), 4.13 (m, 1H), 3.96 (s, 2H), 3.75 (d, 1H, J = 14.6 Hz), 3.58 (q, 2H, J = 13.8 Hz), 3.23 (m, 1H), 2.21 (s, 3H),

1.25 (t, 3H, J = 6.8 Hz), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 185.3 (C^{IV}), 184.5 (C^{IV}), 171.0 (C^{IV}), 151.7 (CH), 149.7 (C^{IV}), 149.2 (C^{IV}), 145.1 (C^{IV}), 144.3 (C^{IV}), 136.7 (C^{IV}), 134.5 (C^{IV}), 133.5 (2CH), 133.0 (C^{IV}), 132.1 (C^{IV}), 132.0 (C^{IV}), 129.1 (2CH), 128.9 (2CH), 128.0 (CH), 126.4 (CH), 126.2 (CH), 124.7 (CH), 122.4 (CH), 117.4 (C^{IV}), 98.4 (CH), 83.8 (C^{IV}), 82.7 (C^{IV}), 70.4 (CH), 70.0 (CH), 69.3 (5CH), 67.6 (CH), 47.7 (CH), 43.1 (CH_2), 40.7 (CH_2), 40.1 (CH_2), 32.0 (CH_2), 21.1 (CH_3), 20.0 (CH_3), 14.2 (CH_3); MS m/z 752 MH^+ ^{37}Cl, 751 $M^{+\cdot}$ ^{37}Cl, 750 MH^+ ^{35}Cl, 749 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2$ $C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 21.4 min, CN-RP 17.2 min.

4.3.11. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-butyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl]phenyl} acetamide (21)

A brown solid was obtained: yield 52%; mp 94 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J = 5.7 Hz), 8.06 (m, 2H), 7.84 (d, 1H, J = 2.1 Hz), 7.68 (m, 2H), 7.64 (d, 1H, J = 9.0 Hz), 7.11 (d, 2H, J = 8.2 Hz), 7.06 (dd, 1H, J = 2.2; 8.5 Hz), 7.03 (d, 2H, J = 8.2 Hz), 6.84 (m, 1H), 6.50 (d, 1H, J = 5.7 Hz), 5.16 (d, 1H, J = 14.2 Hz), 4.45 (dd, 1H, J = 4.7; 14.2 Hz), 4.35 (s, 1H), 4.27 (m, 1H), 4.23 (s, 1H), 4.16 (s, 5H), 4.15 (s, 1H), 3.97 (s, 2H), 3.76 (d, 1H, J = 14.2 Hz), 3.58 (dd, 2H, J = 15.4; 25.3 Hz), 3.23 (m, 2H), 2.22 (s, 3H), 1.56 (m, 2H), 1.35 (m, 2H), 0.87 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 184.3 (C^{IV}), 183.5 (C^{IV}), 170.0 (C^{IV}), 150.4 (CH), 148.8 (C^{IV}), 147.9 (C^{IV}), 144.1 (C^{IV}), 143.3 (C^{IV}), 135.7 (C^{IV}), 133.6 (C^{IV}), 132.5 (2CH), 131.9 (C^{IV}), 131.0 (2C^{IV}), 128.1 (2CH), 127.8 (2CH), 126.7 (CH), 125.3 (CH), 125.2 (CH), 123.7 (CH), 121.4 (CH), 116.3 (C^{IV}), 97.3 (CH), 82.7 (C^{IV}), 81.6 (C^{IV}), 69.4 (CH), 68.9 (CH), 68.3 (5CH), 66.6 (CH), 46.7 (CH_2), 42.1 (CH_2), 39.6 (CH_2), 39.0 (CH_2), 31.0 (CH_2), 29.5 (CH_2), 19.0 (CH_2), 12.8 (CH_3), 12.3 (CH_3); MS m/z 766 MH^+ ^{37}Cl, 765 $M^{+\cdot}$ ^{37}Cl, 764 MH^+ ^{35}Cl, 763 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8Fe$ $CH_2)^+$; HPLC t_R C18 Nucleosil 22.1 min, CN-RP 17.6 min.

4.3.12. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-*N*-isobutyl-2-{4-[(3-methyl-1,4-naphtoquinolyl)methyl] phenyl}acetamide (22)

A brown solid was obtained: yield 36%; mp 80 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, J = 5.3 Hz), 7.98 (m, 2H), 7.80 (d, 1H, J = 2.0 Hz), 7.60 (m, 2H), 7.53 (d, 1H, J = 8.6 Hz), 6.95 (dd, 1H, J = 8.1; 32.3 Hz), 6.44 (d, 1H, J = 5.3 Hz), 5.07 (d, 1H, J = 14.2 Hz), 4.35 (dd, 1H, J = 3.9; 14.2 Hz), 4.26 (m, 1H), 4.16 (m, 2H), 4.09 (s, 5H), 4.05 (d, 2H, J = 7.1 Hz), 3.88 (s, 2H), 3.75 (d, 1H, J = 14.2 Hz), 3.53 (d, 2H, J = 16.0 Hz), 3.05 (m, 2H), 2.14 (s, 3H), 1.19 (t, 2H, J = 7.1 Hz), 0.88 (t, 3H, J = 6.6 Hz), 0.79 (t, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 185.5 (C^{IV}), 184.7 (C^{IV}), 171.6 (C^{IV}), 151.3 (CH), 150.1 (C^{IV}), 148.7 (C^{IV}), 145.3 (C^{IV}), 144.5 (C^{IV}), 136.8 (C^{IV}), 134.9 (C^{IV}), 133.6 (CH), 133.2 (CH), 132.2 (C^{IV}), 132.1 (2C^{IV}), 129.2 (2CH), 129.0 (2CH), 127.7 (CH), 126.5 (CH), 126.4 (CH), 125.0 (CH), 122.6 (CH), 117.4 (C^{IV}), 98.4 (CH), 83.7 (C^{IV}), 82.7 (C^{IV}), 70.3 (CH), 70.0 (CH), 69.5 (5CH), 67.8 (CH), 54.9 (CH_2), 43.3 (CH_2), 40.9 (CH_2), 32.2 (CH_2), 27.5 (CH), 20.5 (CH_3), 19.9 (CH_3), 13.5 (CH_3); MS m/z 766 MH^+ ^{37}Cl, 765 $M^{+\cdot}$ ^{37}Cl, 764 MH^+ ^{35}Cl, 763 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNH$ $CH_2C_{10}H_8$ $FeCH_2)^+$; HPLC t_R C18 Nucleosil 21.9 min, CN-RP 17.5 min; HRMS m/z calcd for $C_{45}H_{43}ClFeN_3O_3$ $(M+H)^+$ 764.2342, found 764.2337.

4.3.13. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl]-ferrocenyl}-*N*-methylbenzamide (23)

A yellow solid was obtained: yield 58%; mp 194 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, 1H, J = 5.3 Hz), 7.77 (d, 1H, J = 1.7 Hz), 7.54 (d, 1H, J = 9.0 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.00 (d, 2H, J = 8.3 Hz), 6.87 (dd, 1H, J = 1.7; 8.9 Hz), 6.68 (m, 1H), 6.47 (d, 1H, J = 5.4 Hz), 5.49 (d, 1H, J = 14.2 Hz), 4.50 (dd, 1H, J = 5.3; 15.7 Hz), 4.35 (m, 1H), 4.27 (d, 1H, J = 6.5 Hz), 4.22 (m, 1H), 4.13 (s, 5H), 4.11 (m, 1H), 3.67 (d, 1H, J = 14.2 Hz), 2.84 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4 (C^{IV}), 151.8 (CH), 149.6 (C^{IV}), 149.3 (C^{IV}), 134.7 (C^{IV}), 134.5 (C^{IV}), 131.6 (2CH), 128.6 (2CH), 128.2 (CH), 124.6 (CH), 124.3 (C^{IV}), 122.3 (CH), 117.3 (C^{IV}), 98.5 (CH), 84.3 (C^{IV}), 81.5 (C^{IV}), 71.0 (CH), 70.5 (CH), 69.3 (5CH), 67.7 (CH), 45.6 (CH_2), 40.6 (CH_2), 37.5 (CH_3); MS m/z 605 MH^+ ^{37}Cl, 604 $M^{+\cdot}$ ^{37}Cl, 603 MH^+ ^{35}Cl, 602 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8$ $FeCH_2)^+$; HPLC t_R C18 Nucleosil 18.4 min, CN-RP 16.3 min.

4.3.14. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl] ferrocenyl}-*N*-ethylbenzamide (24)

A yellow solid was obtained: yield 42%; mp 184 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, 1H, J = 5.3 Hz), 7.80 (d, 1H, J = 1.4 Hz), 7.61 (d, 1H, J = 9.0 Hz), 7.37 (td, 2H, J = 2.1; 8.4 Hz), 6.99 (d, 2H, J = 8.0 Hz), 6.68 (m, 1H), 6.48 (d, 1H, J = 5.4 Hz), 5.26 (d, 1H, J = 14.0 Hz), 4.48 (dd, 1H, J = 4.1; 14.3 Hz), 4.33 (m, 1H), 4.26 (d, 1H, J = 7.8 Hz), 4.21 (m, 1H), 4.13 (s, 5H), 4.12 (m, 1H), 3.85 (d, 1H, J = 14.0 Hz), 3.16 (d, 2H, J = 6.3 Hz), 1.03 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 170.9 (C^{IV}), 151.9 (CH), 149.6 (C^{IV}), 149.4 (C^{IV}), 135.1 (C^{IV}), 134.7 (C^{IV}), 131.7 (2CH), 128.4 (2CH), 127.9 (CH), 124.7 (CH), 123.9 (C^{IV}), 122.3 (CH), 117.3 (C^{IV}), 98.5 (CH), 84.3 (C^{IV}), 82.2 (C^{IV}), 70.7 (CH), 70.3 (CH), 69.4 (5CH), 67.7 (CH), 43.0 (CH_2), 41.5 (CH_2), 40.8 (CH_2), 13.6 (CH_3); MS m/z 619 MH^+ ^{37}Cl, 618 $M^{+\cdot}$ ^{37}Cl, 617 MH^+ ^{35}Cl, 616 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 18.9 min, CN-RP 16.5 min.

4.3.15. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl] ferrocenyl}-*N*-propylbenzamide (25)

A yellow solid was obtained: yield 42%; mp 150 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, 1H, J = 5.3 Hz), 7.80 (m, 1H), 7.56 (d, 1H, J = 8.9 Hz), 7.37 (d, 2H, J = 8.3 Hz), 6.96 (d, 2H, J = 8.3 Hz), 6.91 (m, 1H), 6.64 (m, 1H), 6.47 (d, 1H, J = 5.2 Hz), 5.29 (d, 1H, J = 15.2 Hz), 4.47 (dd, 1H, J = 4.3; 14.5 Hz), 4.32 (m, 1H), 4.25 (d, 1H, J = 7.3 Hz), 4.20 (m, 1H), 4.13 (s, 5H), 4.11 (m, 1H), 3.83 (d, 1H, J = 15.2 Hz), 3.06 (t, 2H, J = 7.3 Hz), 1.48 (m, 2H), 0.67 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 171.1 (C^{IV}), 151.8 (CH), 149.6 (C^{IV}), 149.3 (C^{IV}), 135.1 (C^{IV}), 134.8 (C^{IV}), 131.6 (2CH), 128.3 (2CH), 128.2 (CH), 124.7 (CH), 123.9 (C^{IV}), 122.3 (CH), 117.3 (C^{IV}), 98.5 (CH), 84.0 (C^{IV}), 82.2 (C^{IV}), 70.7 (CH), 70.3 (CH), 69.4 (5CH), 67.7 (CH), 49.9 (CH_2), 41.7 (CH_2), 40.8 (CH_2), 21.3 (CH_2), 11.0 (CH_3); MS m/z 633 MH^+ ^{37}Cl, 632 $M^{+\cdot}$ ^{37}Cl, 631 MH^+ ^{35}Cl, 630 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHC$ H_2C_{10} $H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.3 min, CN-RP 16.6 min; HRMS m/z calcd for $C_{31}H_{30}BrClFeN_3O$ $(M+H)^+$ 630.0610, found 630.0610.

4.3.16. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl] ferrocenyl}-*N*-isopropylbenzamide (26)

A yellow solid was obtained: yield 27%; mp 81 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J = 5.4 Hz), 7.93 (d, 1H, J = 9.0 Hz), 7.88 (d, 1H, J = 2.1 Hz), 7.53 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.5 Hz), 7.12 (dd, 1H, J = 2.0; 9.2 Hz), 6.53 (d, 1H, J = 5.5 Hz), 4.62 (d, 1H, J = 14.2 Hz), 4.45 (d, 1H, J = 13.9 Hz), 4.34 (m, 1H), 4.28 (m, 2H), 4.20 (s, 5H), 4.16 (t, 1H, J = 2.5 Hz), 4.12 (d, 1H, J = 14.2 Hz), 4.03 (m, 1H), 1.36 (d, 1H, J = 6.7 Hz), 1.14 (d, 1H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 170.6 (C^{IV}), 151.9 (CH), 150.0 (C^{IV}), 149.3 (C^{IV}), 135.4 (C^{IV}), 134.7 (C^{IV}), 131.8 (2CH), 128.1 (3CH), 124.7 (CH), 124.1 (C^{IV}), 122.9 (CH), 117.6 (C^{IV}), 98.5 (CH), 84.3 (C^{IV}), 82.9 (C^{IV}), 69.8 (CH), 69.5 (CH), 69.4 (5CH), 67.6 (CH), 53.5 (CH_2), 50.8 (CH_2), 41.5 (CH), 22.0 (CH_3), 21.3 (CH_3); MS m/z 633 MH^+ ^{37}Cl, 632 $M^{+\cdot}$ ^{37}Cl, 631 MH^+ ^{35}Cl, 630 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNH$ $CH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2$ $C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 20.1 min, CN-RP 16.7 min.

4.3.17. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl] ferrocenyl}-*N*-butylbenzamide (27)

A yellow solid was obtained: yield 64%; mp 170 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.50 (d, 1H, J = 5.3 Hz), 7.87 (m, 1H), 7.63 (d, 1H, J = 9.0 Hz), 7.42 (d, 2H, J = 6.7 Hz), 7.03 (d, 2H, J = 8.2 Hz), 6.98 (m, 1H), 6.70 (m, 1H), 6.55 (d, 1H, J = 5.2 Hz), 5.36 (d, 1H, J = 14.0 Hz), 4.55 (dd, 1H, J = 4.6; 14.5 Hz), 4.40 (m, 1H), 4.33 (d, 1H, J = 7.7 Hz), 4.27 (m, 1H), 4.21 (s, 5H), 4.18 (m, 1H), 3.90 (d, 1H, J = 14.0 Hz), 3.17 (m, 2H), 2.33 (m, 1H), 1.50 (m, 2H), 1.13 (m, 2H), 0.80 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 170.9 (C^{IV}), 151.8 (CH), 149.5 (C^{IV}), 149.3 (C^{IV}), 135.1 (C^{IV}), 134.8 (C^{IV}), 131.6 (2CH), 128.3 (2CH), 128.1 (CH), 124.7 (CH), 123.8 (C^{IV}), 122.3 (CH), 117.2 (C^{IV}), 98.5 (CH), 84.0 (C^{IV}), 82.2 (C^{IV}), 70.7 (CH), 70.4 (CH), 69.4 (5CH), 67.7 (CH), 48.0 (CH_2), 41.8 (CH_2), 40.8 (CH_2), 30.2 (CH_2), 19.7 (CH_2), 13.6 (CH_3); MS *m/z* 647 MH^+ ^{37}Cl, 646 $M^{+\cdot}$ ^{37}Cl, 645 MH^+ ^{35}Cl, 644 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.7 min, CN-RP 16.8 min.

4.3.18. 4-Bromo-*N*-{3-[(7-chloroquinolin-4-ylamino)methyl] ferrocenyl}-*N*-isobutylbenzamide (28)

A yellow solid was obtained: yield 31%; mp 142 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, J = 4.0 Hz), 7.77 (m, 1H), 7.43 (d, 1H, J = 8.7 Hz), 7.29 (d, 2H, J = 7.1 Hz), 6.90 (d, 2H, J = 7.2 Hz), 6.80 (d, 1H, J = 8.7 Hz), 6.52 (m, 1H), 6.45 (d, 1H, J = 4.1 Hz), 5.35 (d, 1H, J = 14.4 Hz), 4.45 (dd, 1H, J = 3.0; 14.3 Hz), 4.32 (m, 1H), 4.23 (d, 1H, J = 6.7 Hz), 4.17 (m, 1H), 4.12 (s, 6H), 3.80 (d, 1H, J = 14.3 Hz), 2.96 (m, 2H), 1.88 (m, 1H), 0.71 (d, 3H, J = 5.8 Hz), 0.64 (t, 3H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 171.6 (C^{IV}), 151.8 (CH), 149.5 (C^{IV}), 149.2 (C^{IV}), 135.1 (C^{IV}), 134.7 (C^{IV}), 131.5 (2CH), 128.6 (2CH), 128.2 (CH), 124.6 (CH), 123.7 (C^{IV}), 122.2 (CH), 117.1 (C^{IV}), 98.5 (CH), 83.9 (C^{IV}), 82.1 (C^{IV}), 70.8 (CH), 70.3 (CH), 69.4 (5CH), 67.7 (CH), 55.2 (CH_2), 41.3 (CH_2), 40.79 (CH_2), 26.4 (CH), 20.1 (CH_3), 19.4 (CH_3); MS *m/z* 647 MH^+ ^{37}Cl, 646 M^+ ^{37}Cl, 645 MH^+ ^{35}Cl, 644 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.5 min, CN-RP 16.7 min.

4.3.19. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-methylbenzamide (29)

A yellow solid was obtained: yield 33%; mp 212 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, J = 5.4 Hz), 7.75 (m, 1H), 7.54 (d, 1H, J = 9.0 Hz), 7.12 (d, 2H, J = 8.5 Hz), 6.82 (m, 1H), 6.70 (dd, 2H, J = 2.3; 8.7 Hz), 6.46 (d, 1H, J = 6.5 Hz), 5.53 (d, 1H, J = 15.2 Hz), 4.51 (d, 1H, J = 13.0 Hz), 4.32 (m, 1H), 4.21 (m, 2H), 4.12 (s, 5H), 4.09 (m, 1H), 3.73 (s, 3H), 3.63 (d, 1H, J = 15.2 Hz), 2.89 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.3 (C^{IV}), 160.8 (C^{IV}), 151.5 (CH), 149.6 (C^{IV}), 149.0 (C^{IV}), 134.5 (C^{IV}), 129.1 (2CH), 127.8 (CH), 127.6 (CH), 124.3 (C^{IV}), 122.5 (CH), 117.2 (C^{IV}), 113.4 (2CH), 98.3 (CH), 83.9 (C^{IV}), 81.7 (C^{IV}), 71.0 (CH), 70.5 (CH), 69.2 (5CH), 67.6 (CH), 55.3 (CH_3), 45.6 (CH_2), 40.4 (CH_2), 37.8 (CH_3); MS *m/z* 556 MH^+ ^{37}Cl, 555 $M^{+\cdot}$ ^{37}Cl, 554 MH^+ ^{35}Cl, 553 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 17.9 min, CN-RP 16.1 min.

4.3.20. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-ethylbenzamide (30)

A yellow solid was obtained: yield 34%; mp 158 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, 1H, J = 4.5 Hz), 7.79 (m, 1H), 7.62 (d, 1H, J = 8.7 Hz), 7.53 (d, 2H, J = 7.5 Hz), 6.92 (d, 2H, J = 9.3 Hz), 6.85 (m, 1H), 6.72 (d, 2H, J = 6.9 Hz), 6.47 (d, 1H, J = 4.6 Hz), 5.29 (d, 1H, J = 15.5 Hz), 4.49 (m, 1H), 4.31 (m, 1H), 4.21 (m, 2H), 4.12 (s, 5H), 4.10 (m, 1H), 3.83 (d, 1H, J = 15.5 Hz), 3.73 (s, 3H), 3.25 (m, 2H), 1.06 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 172.0 (C^{IV}), 160.7 (C^{IV}), 151.7 (CH), 149.7 (C^{IV}), 149.2 (C^{IV}), 134.6 (C^{IV}), 128.3 (2CH), 128.2 (CH), 128.0 (CH), 124.7 (C^{IV}), 122.6 (CH), 117.3 (C^{IV}), 113.7 (2CH), 98.4 (CH), 84.0 (C^{IV}), 82.5 (C^{IV}), 70.7 (CH), 70.4 (CH), 69.3 (5CH), 67.6 (CH), 55.4 (CH_3), 45.9 (CH_2), 41.6 (CH_2), 40.8 (CH_2), 13.7 (CH_3); MS *m/z* 570 MH^+ ^{37}Cl, 569 $M^{+\cdot}$ ^{37}Cl, 568 MH^+ ^{35}Cl, 567 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 18.3 min, CN-RP 16.1 min.

4.3.21. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-propylbenzamide (31)

A yellow solid was obtained: yield 35%; mp 150 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, 1H, J = 5.0 Hz), 7.77 (m, 1H), 7.56 (d, 1H, J = 8.9 Hz), 7.06 (d, 2H, J = 7.7 Hz), 6.86 (d, 1H, J = 6.7 Hz), 6.80 (m, 1H), 6.69 (d, 1H, J = 7.7 Hz), 6.45 (d, 1H, J = 4.8 Hz), 5.32 (d, 1H, J = 14.6 Hz), 4.48 (m, 1H), 4.35 (m, 1H), 4.30 (m, 1H), 4.19 (m, 2H), 4.11 (s, 6H), 3.80 (m, 1H), 3.72 (s, 3H),3.13 (m, 2H), 1.49 (m, 2H), 0.68 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 171.2 (C^{IV}), 159.7 (C^{IV}), 150.7 (CH), 148.6 (C^{IV}), 148.2 (C^{IV}), 133.5 (C^{IV}), 127.4 (2CH), 127.3 (CH), 127.0 (CH), 123.5 (C^{IV}), 121.5 (CH), 116.3 (C^{IV}), 112.6 (2CH), 97.4 (CH), 82.9 (C^{IV}), 81.5 (C^{IV}), 69.7 (CH), 69.3 (CH), 68.3 (5CH), 66.5 (CH), 59.4 (CH_3), 54.3 (CH_2), 39.7 (CH_2), 20.3 (CH_2), 13.4 (CH_2), 10.0 (CH_3); MS *m/z* 584 MH^+ ^{37}Cl, 583 $M^{+\cdot}$ ^{37}Cl, 582 MH^+ ^{35}Cl, 581 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 18.7 min, CN-RP 16.4 min; HRMS *m/z* calcd for $C_{32}H_{33}ClFeN_3O_2$ $(M+H)^+$ 582.1611, found 582.1595.

4.3.22. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-isopropylbenzamide (32)

A yellow solid was obtained: yield 42%; mp 203 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (s, 1H), 7.93 (d, 1H, J = 9.2 Hz), 7.82 (s, 1H), 7.46 (m, 1H), 7.24 (m, 2H), 7.03 (d, 1H, J = 8.2 Hz), 6.82 (m, 2H), 6.45 (s, 1H), 4.55 (dd, 1H, J = 3.5; 14.6 Hz), 4.38 (d, 1H, J = 12.8 Hz), 4.26 (m, 1H), 4.21 (m, 1H), 4.17 (m, 1H), 4.11 (s, 6H), 4.04 (m, 1H), 3.75 (s, 3H), 1.31 (d, 3H, J = 7.5 Hz), 1.18 (m, 1H), 1.05 (d, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 170.5 (C^{IV}), 159.9 (C^{IV}), 150.5 (CH), 149.3 (C^{IV}), 147.8 (C^{IV}), 133.7 (C^{IV}), 127.6 (2CH), 127.5 (CH), 126.6 (CH), 123.6 (C^{IV}), 122.3 (CH), 116.5 (C^{IV}), 112.8 (2CH), 97.3 (CH), 83.6 (C^{IV}), 81.8 (C^{IV}), 68.7 (CH), 68.5 (CH), 68.3 (5CH), 66.4 (CH), 54.3 (CH_3), 49.9 (CH_2), 40.5 (CH_2), 39.5 (CH), 21.0 (CH_3), 20.2 (CH_3); MS *m/z* 584 MH^+ ^{37}Cl, 583 $M^{+\cdot}$ ^{37}Cl, 582 MH^+ ^{35}Cl, 581 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNHC\ H_2C_{10}\ H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.2 min, CN-RP 16.5 min.

4.3.23. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-butylbenzamide (33)

A yellow solid was obtained: yield 77%; mp 108 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, 1H, J = 5.4 Hz), 7.78 (d, 1H, J = 1.4 Hz), 7.56 (d, 1H, J = 9.0 Hz), 7.06 (d, 2H, J = 8.3 Hz), 6.86 (d, 1H, J = 7.4 Hz), 6.79 (m, 1H), 6.70 (dd, 2H, J = 2.3; 8.7 Hz), 6.46 (d, 1H, J = 5.5 Hz), 5.31 (d, 1H, J = 14.2 Hz), 4.48 (m, 1H), 4.31 (m, 1H), 4.19 (m, 2H), 4.12 (s, 5H), 4.09 (m, 1H), 3.80 (d, 1H, J = 14.2 Hz), 3.72 (s, 3H), 3.17 (m, 2H), 1.44 (m, 2H), 1.13 (m, 2H), 0.73 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 171.0 (C^{IV}), 159.5 (C^{IV}), 150.7 (CH), 148.6 (C^{IV}), 148.2 (C^{IV}), 133.5 (C^{IV}), 127.5 (2CH), 127.3 (CH), 127.0 (CH), 123.6 (C^{IV}), 121.5 (CH), 116.3 (C^{IV}), 112.5 (2CH), 97.4 (CH), 82.9 (C^{IV}), 81.6 (C^{IV}), 69.7 (CH), 69.3 (CH), 68.3 (5CH), 66.5 (CH), 54.3 (CH_3), 47.2 (CH_2), 40.9 (CH_2), 39.7 (CH), 29.1 (CH_3), 18.7 (CH_2), 12.6 (CH_3); MS *m/z* 598 MH^+ ^{37}Cl, 597 $M^{+\cdot}$ ^{37}Cl, 596 MH^+ ^{35}Cl, 595 $M^{+\cdot}$ ^{35}Cl, 391 $(C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 $(C_9H_5N^{35}ClNH\ CH_2C_{10}H_8\ FeCH_2)^+$; HPLC t_R C18 Nucleosil 19.1 min, CN-RP 16.4 min.

4.3.24. *N*-{3-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-4-methoxy-*N*-isopropylbenzamide (34)

A yellow solid was obtained: yield 35%; mp 191 ± 1 °C; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, 1H, J = 4.9 Hz), 7.76 (m, 1H), 7.43 (d, 1H, J = 9.0 Hz), 6.99 (d, 2H, J = 5.9 Hz), 6.73 (m, 1H), 6.64 (d, 2H,

J = 8.3 Hz), 6.44 (d, 1H, J = 3.6 Hz), 5.41 (d, 1H, J = 14.3 Hz), 4.54 (m, 1H), 4.30 (m, 1H), 4.17 (m, 2H), 4.11 (s, 6H), 3.76 (d, 1H, J = 14.3 Hz), 3.71 (s, 3H), 3.05 (m, 2H), 1.90 (m, 1H), 0.72 (d, 3H, J = 6.5 Hz), 0.65 (t, 3H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 172.5 (C^{IV}), 160.5 (C^{IV}), 151.4 (CH), 149.8 (C^{IV}), 148.8 (C^{IV}), 134.6 (C^{IV}), 128.9 (2CH), 128.3 (CH), 127.6 (CH), 124.5 (C^{IV}), 122.5 (CH), 117.1 (C^{IV}), 113.5 (2CH), 98.4 (CH), 83.8 (C^{IV}), 82.5 (C^{IV}), 70.8 (CH), 70.3 (CH), 69.3 (5CH), 67.5 (CH), 60.4 (CH_3), 55.3 (CH_2), 41.5 (CH_2), 40.8 (CH), 26.4 (CH_2), 20.1 (CH_3), 19.4 (CH_3); MS m/z 598 MH^+ ^{37}Cl, 597 $M^{+\cdot}$ ^{37}Cl, 596 MH^+ ^{35}Cl, 595 $M^{+\cdot}$ ^{35}Cl, 391 ($C_9H_5N^{37}ClNHCH_2C_{10}H_8FeCH_2)^+$, 389 ($C_9H_5N^{35}ClNHCH_2C_{10}H_8FeCH_2)^+$; HPLC t_R C18 Nucleosil 18.9 min, CN-RP 16.4 min.

4.4. In vitro antimalarial activity

Plasmodium falciparum CQ-susceptible NF54 and CQ-resistant K1 strains were cultivated in a variation of the medium previously described,[54] consisting of RPMI 1640 supplemented with 0.5% ALBUMAX® II, 25 mM Hepes, 25 mM $NaHCO_3$ (pH 7.3), 0.36 mM hypoxanthine, and 100 μg/mL neomycin. Human erythrocytes served as host cells. Cultures were maintained in an atmosphere of 3% O_2, 4% CO_2, and 93% N_2 in humidified modular chambers at 37 °C. Compounds were dissolved in DMSO (10 mg/mL) and diluted in hypoxanthine-free culture medium. Infected erythrocytes (2.5% hematocrit and 0.3% parasitemia) were added to each drug and titrated in duplicates over a 64-fold range in 96-well plates. After 48 h incubation, 0.5 μCi of [^{3}H]hypoxanthine was added and plates were incubated for an additional 24 h. Parasites were harvested onto glass–fiber filters and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations (IC_{50}) were estimated by linear interpolation.[55]

4.5. β-Hematin inhibition assay

Drug solutions (89.1 mM, 53.5 mM, 26.7 mM, 17.8 mM, 13.4 mM, 8.9 mM, 4.5 mM, 1.8 mM, and 0 mM) were prepared by dissolving the drug in MeOH, 1 M HCl or DMSO. Hematin stock solution (1.68 mM) was prepared by dissolving bovine hemin (0.64 mg) in 0.1 M NaOH (980 μL). The solution was incubated at room temperature for 60 min. In a series of Eppendorf tubes 2.0 μL of 1 M HCl and 2.0 μL of drug solution (or solvent for the blank) were dispensed. The Eppendorf tubes were placed in an incubator at 60 °C and then, 12.9 M sodium acetate solution, pH 5.0, (11.7 μL) preincubated at 60 °C was added. If drugs are dissolved in 1 M HCl, 2.0 μL of MeOH or DMSO, accordingly, were added instead of 1 M HCl. The β-hematin formation process was initiated by addition of hematin stock solution (20.2 μL) prepared above. The final hematin concentration was 1 mM, the final drug concentrations were 5 mM, 3 mM, 1.5 mM, 1 mM, 0.75 mM, 0.5 mM, 0.25 mM, 0.1 mM, and 0 mM and the final solution pH was 4.5. The reaction mixtures were incubated at 60 °C for 60 min. After incubation, the reaction mixtures were quenched at room temperature by adding 900 μL of 200 mM HEPES 5% (v/v) pyridine solution, pH 8.2, to adjust the final pH of the mixtures to a value between 7.2 and 7.5. Then, 20 mM HEPES 5% (v/v) pyridine solution, pH 7.5 (1100 μL) was added. The Eppendorf tubes were shaken and the precipitate of β-hematin was scraped from the walls of the Eppendorf tubes to ensure complete dissolution of hematin. The β-hematin mixtures were allowed to stand at room temperature for at least 15 min. The supernatant was carefully transferred to a cuvette without disturbing the precipitate and absorption was measured at 405 nm.

4.6. GSSG reduction assay with *P. falciparum* glutathione reductase

The standard assay was conducted at 25 °C in a 1 mL-cuvette. The assay mixture contained 100 μM NADPH and 1 mM GSSG in GR buffer (100 mM potassium phosphate buffer, 200 mM KCl, 1 mM EDTA, pH 6.9). IC_{50} values were evaluated in duplicate in the presence of seven inhibitor concentrations ranging from 0 to 100 μM (100 μM, 10 μM, 5 μM, 2.5 μM, 1 μM, 0.5 μM, and 0.1 μM). Inhibitor stock solutions were prepared in 100% DMSO). A final content of 1% DMSO was kept constant in all assays. The reaction was started by adding 6.5 mU *P. falciparum* GR and initial rates of NADPH oxidation were monitored at 340 nm.

4.7. Cleavage of dual molecules under biomimetic conditions

Drug solutions (89.1 mM) were prepared by dissolving the drug in 1 M HCl or DMSO, according the solubility of the drug. Hematin stock solution (1.68 mM) was prepared by dissolving bovine hemin (0.64 mg) in 0.1 M NaOH (980 μL). The solution was incubated at room temperature for 60 min. After incubation, the reaction mixtures were centrifuged and 50 μL of the supernatant was injected in a high pressure liquid chromatography (HPLC) column, Macherey-Nagel C18 Nucleosil column (4 × 300 mm, 5 μm, 100 Å). Analytical HPLC was performed on a Spectra system equipped with a UV detector set at 254 nm. The following solvent systems were used: eluent (A): 0.05% trifluoroacetic acid (TFA) in H_2O, eluent (B) 100% CH_3CN. HPLC retention times (HPLC t_R) were determined at flow rates of 1 mL/min, using the following conditions: 100% eluent A for 5 min, then a gradient run to 100% eluent B over the next 20 min. As reference, the GR inhibitor **M$_5$**, the GSH depletor **10** and the secondary amines were also injected in HPLC under the same conditions.

4.7.1. Cytosolic conditions

In Eppendorf tubes, drug solutions (89.1 mM) were incubated at 37 °C in a phosphate buffer solution, pH 7.4. The reaction mixture was injected in HPLC after 1 h, 17 h and 24 h.

4.7.2. Vacuolar conditions

In Eppendorf tubes, 10 μL of 1 M HCl were incubated at 37 °C and then, 58.5 μL of 12.9 M sodium acetate solution, pH 5.2, preincubated at 60 °C were added. Finally, 100 μL of 1.68 mM hematin stock solution (or 0.1 M NaOH solution for the blank), 10 μL of drug solution (89.1 mM) and 2.5 μL of 5 M H_2O_2 solution were added. The reaction mixture was incubated at 37 °C and the supernatant was injected in HPLC after 1 h.

4.7.3. 4-Amino-7-chloroquinoline

1H NMR (D_2O) δ 8.20 (H2), 8.10 (H5), 7.82 (H8), 7.61 (H6), 6.80 (H3); ^{13}C NMR (D_2O) δ 144.5 (C2), 130.3 (C6), 127.8 (C5), 122.1 (C8), 105.3 (C3); MS m/z 177; HPLC t_R C18 Nucleosil 12.43 min.

4.8. X-ray crystal structure determination of 6 and 29

Suitable crystals were mounted on a Bruker SMART CCD area-detector diffractometer with Mo Kα (λ = 0.71073 Å). The structures were solved by using SHELXS97 and refined by using SHELXL97.[56] All non-hydrogen atoms were anisotropically refined. Hydrogen atoms were introduced in calculated positions in the last refinements and they were allocated one overall isotropic thermal parameter. Major crystallographic data and collection details are summarized in Table SI1. CCDC 652254 and CCDC 652255 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.9. NMR experiments

NMR spectra were recorded at room temperature in $CDCl_3$ on Bruker Avance 300 and at 280 K in $CDCl_3$ on Bruker Avance 400 MHz spectrometers. A drop of H_2O was added on the surface of the sample in order to prevent the evaporation of the solvent and consequently the degradation of field homogeneity during acquisition.[57] ^1H and ^{13}C spectra were referenced to internal TMS or the residual proton signals of the deuterated solvents (δ^1H = 7.24 ppm and δ ^{13}C = 77.2 ppm). ^{15}N were referenced indirectly as described by Wishart et al.[58] COSY, ^{13}C-HSQC, ^{13}C-HMBC, ^{15}N-HSQC and ^{15}N-HMBC experiments were performed using the standard sequences. HMBC experiments used an 8 Hz long range coupling constant. Two-dimensional NOESY experiments were performed with 300 ms mixing time. The UDEFT experiments were recorded as described by Lippens and co-workers typically.[59]

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Supplementary data

Table SI1 showing the Crystallographic data for **29**. Figure SI2 showing the crystal structure of compound **29**. Table SI3 showing ^1H, ^{13}C and ^{15}N chemical shift assignments in $CDCl_3$ at 280 K and ^1H frequency of 400 MHz for the molecule **29**. Figure SI4 showing the definition of H12a, H12b, H23a and H23b. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.008.

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