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Original article

Synthesis and antimalarial activity of carbamate and amide derivatives of 4-anilinoquinoline

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Abstract

A series of 4-anilinoquinolines bearing an amino side chain linked to the aromatic ring with a carbamate or an amide bond were synthesized and evaluated for their antimalarial activity and their cytotoxicity upon MRC-5 cells. Among the 17 compounds, a majority was found to be active in the low nanomolar range against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro with relative low cytotoxicity. Two compounds were then tested on mice infected by *Plasmodium berghei* and were found to exhibit reasonable in vivo activity.

Keywords: Drug design; *Plasmodium falciparum*; Antimalarials; Amodiaquine; 4-Aminoquinolines

1. Introduction

Malaria can be considered actually as one of the most widespread diseases in the world with almost one-half of the world's population exposed to risk of infection and 2 million deaths each year [1]. Chloroquine (CQ, Chart 1) has been the mainstream drug in the fight against *Plasmodium falciparum* since the 1950s, but its efficacy is eroded by the emergence of resistant parasites. Development of drug resistance to CQ in malaria has become a major health concern and has so prompted the re-examination of alternative antimalarials such as amodiaquine (AQ, Chart 1), a 4-aminoquinoline where the alkyl chain of CQ is replaced by an aromatic ring. AQ proved to be effective against CQ-resistant strains [2] and comparative trials of CQ and AQ for the treatment of acute, uncomplicated infections in Gambia, in West and Central Africa and in Nigeria showed that AQ was superior to CQ, displaying lower parasitological and clinical failure rates [3–5]. However, the clinical use of AQ has been severely restricted since the mid-1980s because cases of agranulocytosis and hepatotoxicity were observed with its long term use [6,7]. This AQ toxicity is thought to be due to extensive metabolization of the 4-hydroxyanilino moiety to its quinoneimine variant [8,9]. However, WHO's guidelines for treatment

Abbreviations: AQ, amodiaquine; CQ, chloroquine; DMAP, 4-(dimethtylamino)pyridine; DSC, *N*,*N*'-disuccinimidylcarbonate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (thiazolyl blue); P_{HPLC}, purity determined by HPLC; TLC, thick-layer chromatography; t_R, HPLC retention time; DMAP, 4-dimethylaminopyridine; DSC, *N*,*N*'-disuccinimidylcarbonate.

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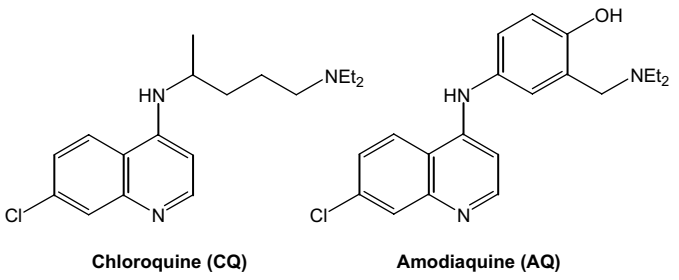


Chloroquine (CQ) **Amodiaquine (AQ)**

Chart 1. Structure of chloroquine and amodiaquine.

still recommend the use of AQ in combination with artemisinin derivatives such as artesunate, named Arsucam®, or if not available with sulfadoxine/pyrimethamine.

We synthesized 4-anilinoquinolines retaining the aromatic ring of AQ but lacking the hydroxyl group responsible for toxicity in order to access compounds as active as AQ and presenting lower cytotoxicity [10,11]. It led notably to compound **1** and its ester or amine derivatives which presented good in vitro antimalarial activity combined to low cytotoxicity and reasonable in vivo antimalarial activity. In order to complete this structure–activity relationships study and optimize the second side chain in this 4-anilinoquinoline series, we present here the synthesis and evaluation of two series of compound **1** derivatives: the carbamate ones **2**–**7** (Chart 2), and the acid or amide ones **8**–**18** (Chart 3).

2. Chemistry

Compounds **2**–**18** were obtained from compound **1**, which was synthesized in a three-step procedure according to Delarue et al (Scheme 1) [10].

Several methods were considered in order to synthesize carbamates **2**–**7**: reaction of alcohol **1** with commercial or *in situ* generated isocyanates (by means of triphosgene, 1,1′-carbonyldiimidazole or Boc_2O/DMAP) [12–15], or activation of alcohol **1** by means of triphosgene, *N*,*N*′-disuccinimidylcarbonate (DSC) or *para*-nitrophenylchloroformate [12,16–18] followed by reaction with an amine. However, only two methods proved to be relatively efficient depending on the desired compound: reaction of alcohol **1** with isocyanates generated by means of triphosgene or activation of this alcohol with DSC. With other methods, starting material was either recovered or degraded.

For compounds **2**–**4**, activation of alcohol **1** was realized thanks to DSC (Scheme 2) [17]. Activated intermediate was then subjected to an amine to give the desired carbamate. Amine was *N*,*N*-dimethylethylenediamine for compound **2** which was obtained with a moderate 24% yield. For compounds **3** and **4**, a two-step procedure was used with reaction of bromoethylamine followed by substitution of the bromine atom by either piperidine or morpholine. It led to compounds **3** and **4** with low to moderate yields (11% and 29% yields, respectively).

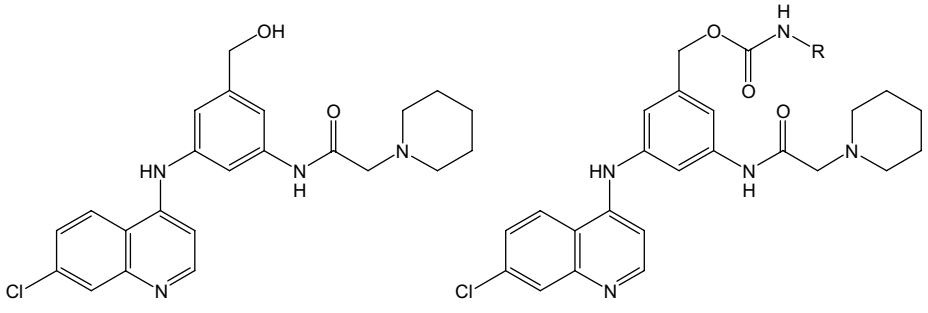
For compounds **5**–**7**, alcohol **1** was subjected to an isocyanate to give the desired carbamates (Scheme 3). This isocyanate was commercial for compound **7** or was generated *in situ* by reaction of the appropriate amine with triphosgene [12] for compounds **5** and **6**. Carbamates **5**–**7** were so obtained in low to moderate yields (33%, 17% and 11% yields, respectively).

Acid derivative **8** was obtained in excellent yield, around 90%, by oxidation of compound **1** using periodic acid with catalytic amount of chromium oxide (Scheme 4) [19]. It was then subjected to various amines with PyBrop as activating agent and diethylamine as base (Scheme 4). This reaction afforded amides **9**–**18** in moderate to good yields, from 11% to 75% yield, primary amines giving lower yields in general than secondary amines.

3. Biological results and discussion

All the compounds were tested for their activity against the CQ-resistant strain FcB1R of *P. falciparum* (CQ: IC_{50} = 126 nM). For compounds **1**–**4** and **16**, activity was also measured against a CQ-sensitive strain Thai (CQ: IC_{50} = 14 nM), and another CQ-resistant strain K1 (CQ: IC_{50} = 183 nM) (Table 1).

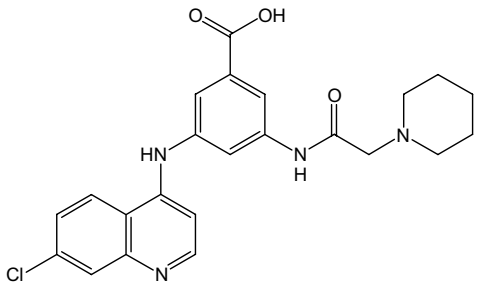
In the carbamate series, all compounds **2**–**7** were found to be at least as active as compound **1**, three presenting IC_{50} around 10 nM (compounds **2**, **3** and **5**). Those compounds



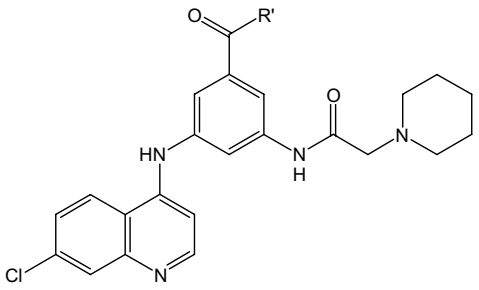
1

2 R = $(CH_2)_2$-NMe_2
3 R = $(CH_2)_2$-piperidine
4 R = $(CH_2)_2$-morpholine
5 R = $(CH_2)_3$-NEt_2
6 R = $(CH_2)_3$-piperazine
7 R = CH_2Ph

Chart 2. General structures of compounds **1**–**7**.



8



9 R' = NH-$(CH_2)_2$-NMe_2
10 R' = -NH-$(CH_2)_3$-NMe_2
11 R' = NMe-$(CH_2)_2$-NMe_2
12 R' = NH-$(CH_2)_2$-pyrrolidine
13 R' = NH-$(CH_2)_2$-morpholine
14 R' = piperidine
15 R' = morpholine
16 R' = *N*-methylpiperazine
17 R' = *N*-phenylpiperazine
18 R' = *N*-benzylpiperazine

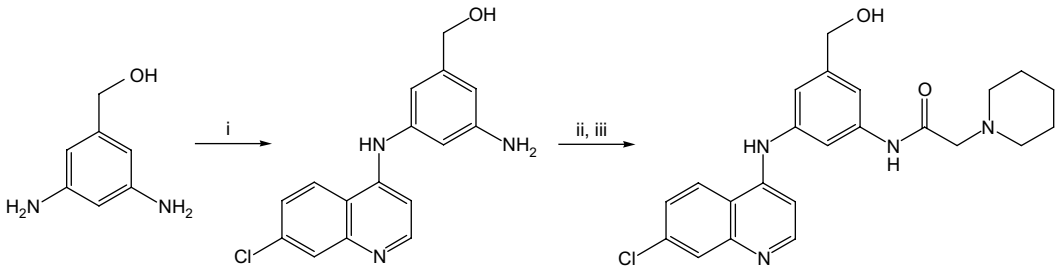
Chart 3. General structures of compounds **8**–**18**.

were active whatever the strain used. On the contrary to compound **1**, which lost a great part of its activity against K1 strain, compounds **3** and **4** improve their IC_{50} against this highly resistant strain. By comparing the influence of the carbamate side chain on activity, we could deduce some structure–activity relationships: (i) presence of an amine on the side chain improved the activity (compound **2**, **3** and **5** versus compound **7**), (ii) the length of the carbon side chain between carbamate bond and terminal amine could comprised two or three methylenes without any influence on activity (compounds **2** and **5** versus compound **6**), (iii) the tertiary amine could be cyclic or not (compounds **2** and **3**), (iv) presence of another electronegative atom on the side chain caused a small decrease in activity (compounds **4** and **6**, Chart 2).

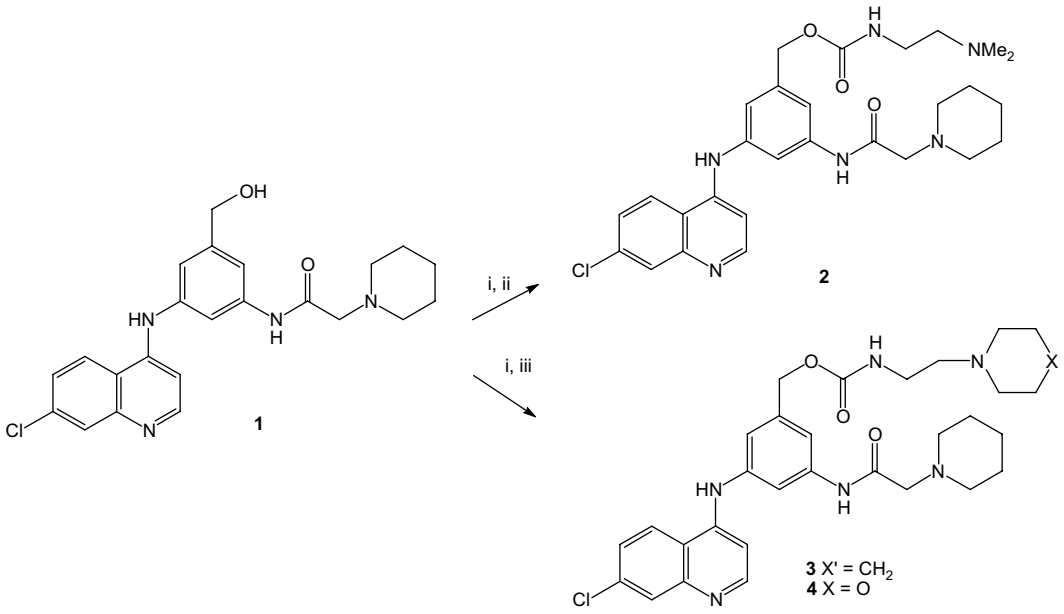
Acid derivative **8** was found to display no antimalarial activity. This lack of activity is not surprising and could be explained by a bad cellular penetration due to the acidic function.

In the amide series, five compounds were found to be at least as active as compound **1**, with IC_{50} between 17 and 50 nM (compounds **9**, **11**, **12**, **16** and **18**, Chart 3). Compound **16** was active whatever the strain used (with a slight improvement against FcB1R strain) as for compounds **2**–**4** in the carbamate series. Some structure–activity relationships could be deduced in the amide series: (i) presence of an amine on the side chain is required to improve activity (compounds **9**, **11**–**13** and **16**–**18** versus compounds **14** and **15**), (ii) the length of the carbon side chain between amide and terminal amine must be of two methylenes but not three (compound **9** versus compound **10**), (iii) the terminal amine could be cyclic or not but slightly higher activity was observed for cyclic amine (compounds **9** and **12**), (iv) presence of another electronegative atom on the side chain caused a small decrease in activity (compounds **13**), (v) tertiary amide was preferred to secondary amide (compounds **9** and **11**).

The relationships in the amide and carbamate series are very similar except for the chain length which is crucial for the amide derivatives but not for the carbamate ones. It can be assumed that terminal amine in those side chains must be separated from the aromatic moiety by 4, 6 or 7 methylene units but not 5 (compounds **9** and **2** versus compound **10**). This is confirmed by previous results observed in the ester series [10] (compounds **19**–**24**, Chart 4, Table 2) or in the amine series [10] (compounds **25**–**29**, Chart 4, Table 2). In those cases a slight decrease of activity was observed when terminal amine was separated by 5 methylene units from the aromatic moiety compared to molecules presenting separation of 4, 6 and 7 methylene units (compound **20** versus compounds **19**, **21** and **22** for ester series, compound **26** versus compound

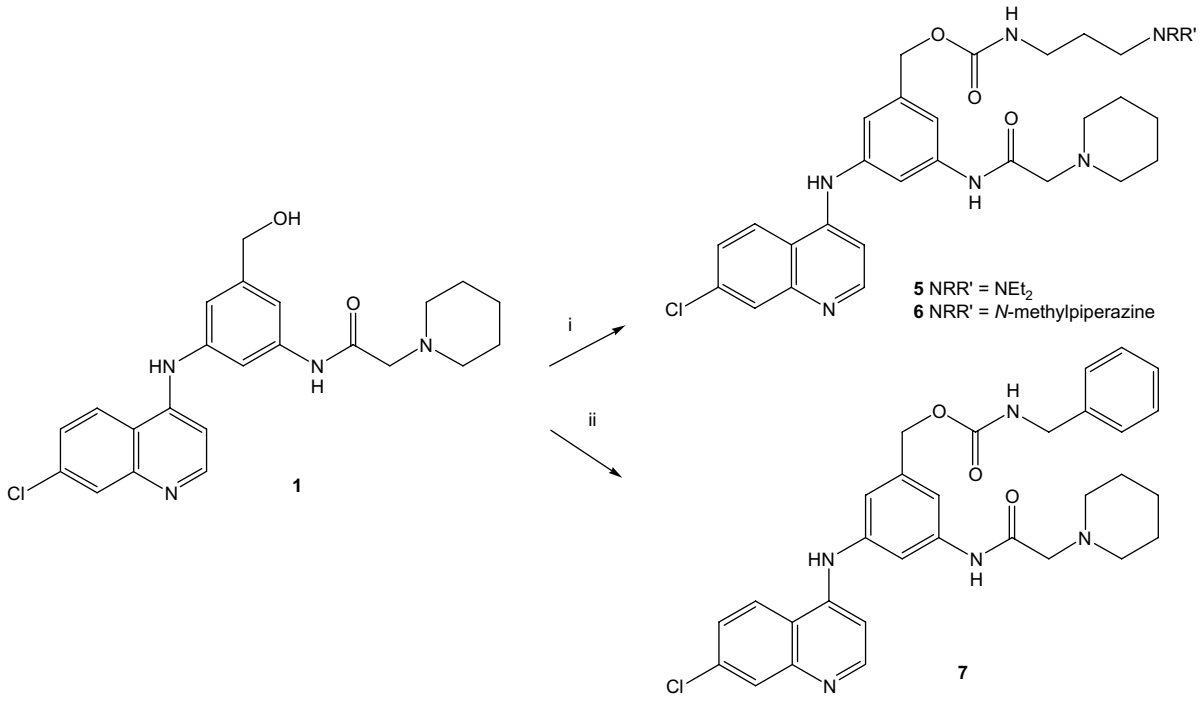


Scheme 1. Synthesis of compound **1**. Reagents: (i) 4,7-dichloroquinoline, *N*-methylmorpholine, EtOH/$CHCl_3$ 9/1, rt, 48 h; (ii) chloroacetic acid, *N*-ethylpiperidine, PyBroP, DMF, rt, 7 h; (iii) 4-methylpiperidine, reflux, 5 h.

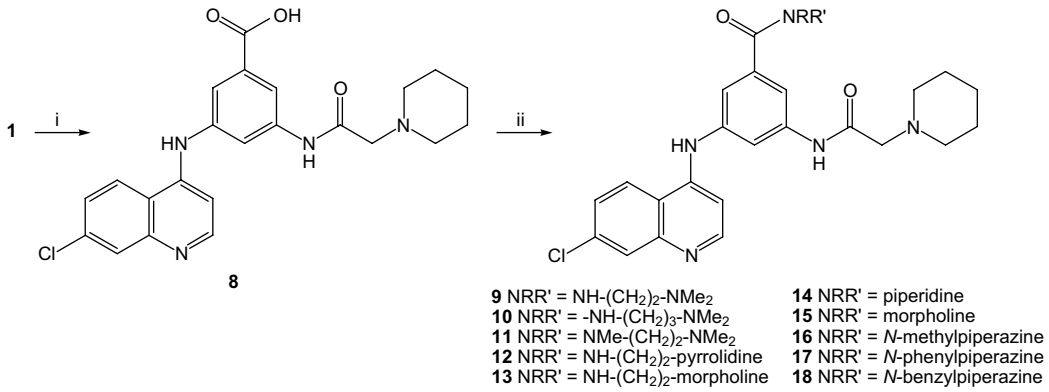


Scheme 2. Synthesis of compounds **2**–**4**. Reagents: (i) DSC, pyridine, DCM, rt, overnight; (ii) *N*,*N*-dimethylethylenediamine, DMAP, rt, overnight; (iii) bromoethylamine bromhydrate, DMAP, rt, overnight then piperidine for compound **3** or morpholine for compound **4**, DCM, reflux, overnight.

25 for amine series). There exist, however, two major differences between carbamate/amide and ester/amine series (Table 2): (i) in the ester series, cyclic amine was found to be highly superior to the acyclic ones (compounds **21** and **24** versus compound **23**), result not observed in the carbamate/amide/amine series (compounds **2** and **3** in the carbamate series, compounds **9** and **12** in the amide series, compounds **25**, **26** and **27** in the amine series), (ii) no real influence of an additional electronegative atom was observed in the ester/amine series (compounds **21** and **24** in the ester series, compounds **28** and **29** in the amine series), while in the carbamate/amide series, introduction of an additional electronegative atom caused decrease of activity (compound **4** versus compound **3** in the carbamate series, compound **13** versus **12** in the amide series).



Scheme 3. Synthesis of compounds **5**–**7**. Reagents: (i) 3-diethylaminopropylisocyanate (for compound **5**) or 3-(4-methylpiperazino)-propylisocyanate (for compound **6**), DCM/DMF 10/1, rt, overnight; (ii) benzylisocyanate, DCM/DMF 10/1, rt, overnight.



Scheme 4. Synthesis of compounds **8**–**18**. Reagents: (i) periodic acid, CrO_3 cat., MeCN, rt, 2 h; (ii) HNRR′, PyBroP, DIEA, DCM/DMF 10/1, rt, overnight.

The cytotoxicity of the different compounds was then evaluated upon human MRC-5 cells (diploid embryonic lung cell line) (Table 1) and expressed as a CC_{50} which is the concentration of drug causing 50% cytotoxicity. In the carbamate and amide series, we could observe in general a slight decrease in cytotoxicity compared to compound **1** with the exception of compounds **2**, **5** and **6**. For the amide and the carbamate series, on the contrary to the ester series, morpholine substituent did not decrease cytotoxicity (Table 2).

All the derivatives revealed a selectivity index (CC_{50}/IC_{50} on FcB1R resistant strain) superior to that of CQ, except compounds **6**, **10**, **14** and **17**. We could remark that, in general, selectivity index is better for carbamate and amide series than for ester and amine series.

Hence it was encouraging to consider compound **3** for further evaluation, which presented the best activity in the carbamate series with good selectivity index (1114). In the amide series compound **16** was preferred to compound **12** which presented, however, a better selectivity index (1024 versus 833) with a similar in vitro activity (around 15 nM). Indeed, compound **12** was hardly obtained with poor yield (16%), while compound **16** was obtained easily with a moderate 32% yield.

Compounds **3** and **16** were so tested in vivo at the concentration of 40 mg/kg/day (Table 3) and were compared to the

Table 1
In vitro antimalarial activity upon three *Plasmodium falciparum* strains, in vitro cytotoxicity of compounds **1**–**18** and selectivity index against FcB1R strain

Compound	IC_{50}[a] (nM)			SI[f]	CC_{50} (μM) upon MRC-5 cells[g]
	Thai	FcB1R	K1		
CQ	14.3 ± 2.4[e]	126 ± 26[e]	183 ± 35[e]	397	50
AQ	4.6 ± 0.8[e]	4.8 ± 0.9[e]	9.4 ± 1.1[e]	2500	12
1	89.6 ± 13.1[c]	44.5 ± 9.7[c]	151.6 ± 48[e]	281	12.5
2	nd	10.2 [b]	nd	304	3.1
3	19.4 ± 6.5[c]	11.2 ± 2.5[d]	9.7 ± 0.8[c]	1116	12.5
4	38.6 ± 4[c]	48.3 [b]	19.4 ± 8.7[c]	>259	>12.5
5	nd	14.5 [b]	nd	434	6.3
6	nd	44 ± 16[c]	nd	143	6.3
7	nd	36 ± 2[c]	nd	>347	>12.5
8	nd	>1000	nd	nd	>32
9	nd	31.4 ± 12.9[c]	nd	478	15
10	nd	181.1 ± 49.5[c]	nd	>177	>32
11	nd	18.8 ± 0.9[c]	nd	851	16
12	nd	16.6 ± 0.8[d]	nd	1024	17
13	nd	58.6 ± 3.4[c]	nd	>546	>32
14	nd	285.6 ± 19.7[c]	nd	>112	>32
15	nd	107.5 ± 22.7[c]	nd	> 298	>32
16	38.4 ± 1.6[c]	19.2 ± 0.7[c]	51.2 ± 8.6[c]	833	16
17	nd	151.1 ± 9.5[c]	nd	106	16
18	nd	50.6 ± 9.0[c]	nd	296	15

nd: not determined.
[a] Parasites were considered resistant to CQ for $IC_{50} > 100$ nM.
[b] Number of experiments: $n = 2$.
[c] Number of experiments: $n = 3$.
[d] Number of experiments: $n = 4$.
[e] Number of experiments: $n = 6$.
[f] SI: selectivity index (CC_{50} MRC-5/IC_{50} FcB1R).
[g] CC_{50} is the IC_{50} value for cytotoxicity calculated on the basis of three experiments.

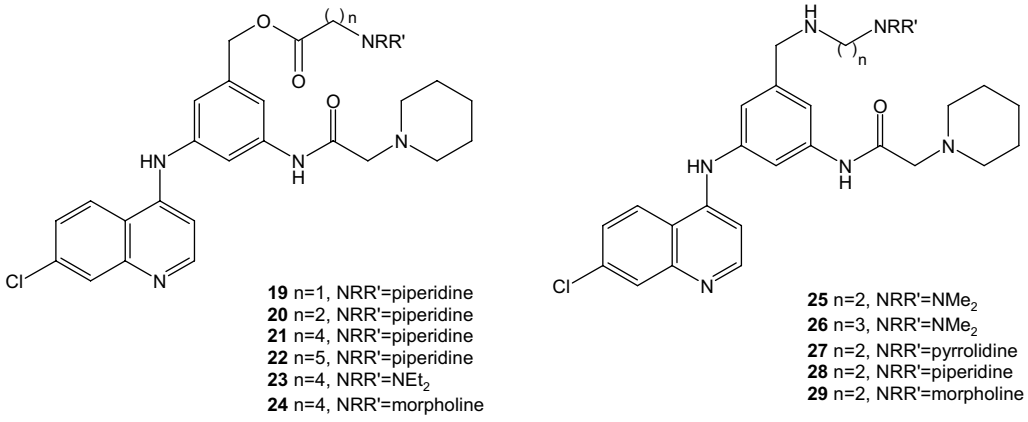


Chart 4. General structures of compounds **19**–**29**.

already known in vivo activity of compound **1**, ester **24** and amine **29** which were the most active of the ester and amine series [10]. Carbamate **3** exhibited an activity in vivo similar to ester **24** (50% and 73% MST, respectively), while amide **16** was more active in vivo than ester **24** and compound **1** with a 150% MST. But those two compounds **3** and **16** did not exhibit better in vivo activity than amine **29**, which was found to cure mice at the same dose. No toxicity was observed in mice.

4. Conclusion

This carbamate and amide series present interesting compounds in term of in vitro antimalarial activity and cytotoxicity and contribute to complete the structure–activity relationships study carried out on 4-anilinoquinoline **1** derivatives. It has been proven that introduction of the amide or carbamate linker improves the efficiency of those derivatives compared to the ester linker in term of selectivity index and in vivo activity. Compound **16** presents the best results in those two series with high in vitro activity whatever the strains used, a good selectivity index and a reasonable activity in vivo.

5. Materials and methods

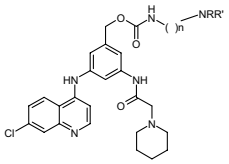
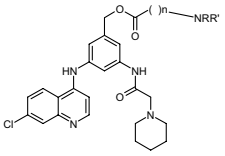
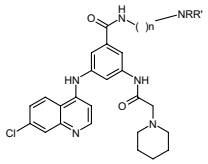
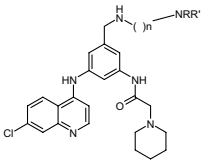
5.1. Chemistry

All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. Chromatography was undertaken using silica gel 60 (230–400 mesh ASTM) from Macherey-Nagel. Thick-layer chromatography (TLC) was performed using silica gel from Merck, from which the compounds were extracted by the following solvent system: CH_2Cl_2/MeOH/NH_4OH 80/20/1. All melting points were determined on a Büchi melting point apparatus and were uncorrected. NMR spectra were obtained using a Bruker 300 MHz spectrometer and chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. Mass spectra were recorded on a MALDI-TOF Voyager-DE STR (Applied Biosystems, Palo Alto, CA) with a trihydroxy-acetophenone matrix. The purity of final compounds **2**–**18** was verified by high pressure liquid chromatography (HPLC) using C18 Vydac (C18 V) column. Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in EtOH and injected through a 50 μL loop. The following eluent systems were used: A (H_2O/TFA 100/0.05) and B (CH_3CN/H_2O/TFA 80/20/0.05). HPLC retention times (t_R) were obtained, at flow rates of 1 mL/min, using the following conditions: a gradient run from 100% eluent A for 5 min, then to 100% eluent B over the next 30 min. 4,7-Dichloroquinoline was obtained from Acros and other reagents from Acros, Aldrich, Avocado and Lancaster. Compound **1** was synthesized according to the procedure described by Delarue et al. [10].

*5.1.1. (2-Dimethylamino-ethyl)-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**2**)*

To a solution of compound **1** (200 mg, 0.47 mmol, 1 eq.) in anhydrous DCM (10 mL) were added, at 0 °C, DSC (483 mg, 1.89 mmol, 4 eq.) and pyridine (152 μL, 1.88 mmol, 4 eq.). After stirring the mixture at room temperature overnight, Et_2O (20 mL) was added and the reactive medium filtered. The precipitate was solubilized in anhydrous DMF (10 mL) with *N*,*N*-dimethylethylenediamine (124 mg, 1.42 mmol, 3 eq.) and DMAP (173 mg, 1.43 mmol, 3 eq.). After further stirring at room temperature overnight, the reactive medium was concentrated and the residue was taken up in DCM (10 mL), the organic layer was washed twice with $NaHCO_3$ 1 M (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated. The residue was finally purified by TLC (DCM/MeOH/NH_4OH 85/15/0.5) to yield compound **2** as a yellow solid (60 mg, 24% yield); R_f 0.30 (DCM/MeOH/NH_4OH 85/15/1); mp = 80 °C; P_{HPLC} 90%; t_R = 14.13 min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.37–1.39 (m, 2H, CH_2), 1.51–1.58 (m, 4H, CH_2), 2.12 (s, 6H, $N(CH_3)_2$), 2.28 (t, J = 6.7 Hz, 2H, CH_2NMe_2), 2.43–2.46 (m, 4H, CH_2), 3.04–3.10 (m, 2H, CH_2NHCO), 3.06 (s, 2H, $COCH_2N$), 4.98 (s, 2H, CH_2Ar), 6.96 (d, J = 5.3 Hz, 1H, Quin-H_3), 7.00 + 7.32 + 7.72 (s, 3H, Arom-H), 7.16 (t, J = 5.7 Hz, 1H, OCONH), 7.56 (dd, J = 9.0 Hz, J = 2.2 Hz, 1H, Quin-H_6),

Table 2
Comparison of in vitro antimalarial activity (nM) and selectivity index against FcB1R strain between the carbamate, ester, amide and amine derivatives

NRR′	Carbamates 				Esters [10] 				Amides 				Amines [10] 			
	Ref	*n*	IC_{50}[a,b]	SI[c]	Ref	*n*	IC_{50}[a,b]	SI[c]	Ref	*n*	IC_{50}[a,b]	SI[c]	Ref	*n*	IC_{50}[a,b]	SI[c]
	**2**	2	10.2	304					**9**	2	31.4	478	**25**	2	15.5	1154
									10	3	181.1	>177	**26**	3	134	174
	**5**	3	14.5	434	**23**	4	141.1	89								
									**12**	2	16.6	1024	**27**	2	10.2	182
					**19**	1	54	231								
	3	2	11.2	1116	**20**	2	78.9	158					**28**	2	6.6	648
					21	4	15.5	806								
					22	5	27.4	456								
	**4**	2	48.3	434	**24**	4	14.1	887	**13**	2	58.6	546	**29**	2	7.9	406

[a] Parasites were considered resistant to CQ for $IC_{50} > 100$ nM.
[b] n, number of experiments between 3 and 6.
[c] SI: selectivity index (CC_{50} MRC-5/IC_{50} FcB1R).

Table 3
Antimalarial activity of compounds **1**, **3**, **16**, **24** and **29** on *P. berghei* in mice

Compound	Dose (mg/kg)	Reduction (%) of parasitaemia, day 4	Excess MST (%)
CQ	10	100	Cured
AQ	10	100	Cured
1	40	100	110
3	40	100	50
16	40	99.96	158
24	40	99.8	73
29	40	100	Cured

Excess MST was defined as the percentage of prolongation of the mean survival time of treated mice compared to that of control mice.

7.89 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.47 (d, $J = 5.4$ Hz, 1H, Quin-H_2), 9.15 (s, 1H, NH), 9.76 (s, 1H, CONH); *m/z* 538.2 ($M^+ + 1$).

5.1.2. General procedure for synthesis of compounds *3* and *4*

To a solution of compound **1** (200 mg, 0.47 mmol, 1 eq.) in anhydrous DCM (10 mL) were added, at 0 °C, DSC (483 mg, 1.89 mmol, 4 eq.) and pyridine (152 μL, 1.88 mmol, 4 eq.). After stirring the mixture at room temperature overnight, Et_2O (20 mL) was added and the reactive medium filtered. The precipitate was solubilized in anhydrous DMF (10 mL) with bromoethylamine bromhydrate (290 mg, 1.42 mmol, 3 eq.) and DMAP (173 mg, 1.43 mmol, 3 eq.). After stirring the mixture at room temperature overnight, a solution of piperidine or morpholine (2.36 mmol, 5 eq.) in DCM (10 mL) was added. After further stirring at reflux overnight, the reactive medium was concentrated and the residue was taken up in DCM (10 mL), the organic layer was washed twice with $NaHCO_3$ 1 M (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated. The residue was finally purified by TLC (DCM/MeOH/NH_4OH 85/15/0.5) to yield the desired compound.

*5.1.2.1. (2-Piperidin-1-yl-ethyl)-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**3**).* Yellow solid (30 mg, 11% yield); R_f 0.20 (DCM/MeOH/NH_4OH 85/15/1); P_{HPLC} 97%, $t_R = 14.51$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.24–1.56 (m, 12H, CH_2), 2.56–2.61 (m, 10H, CH_2), 3.12 (s, 2H, $COCH_2N$), 3.19–3.21 (m, 2H, CH_2NHCO), 5.01 (s, 2H, CH_2Ar), 6.97 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.04 + 7.36 + 7.71 (s, 3H, Arom-H), 7.30 (broad s, 1H, OCONH), 7.57 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.90 (d, $J = 2.3$ Hz, 1H, Quin-H_8), 8.44 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.48 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.18 (s, 1H, NH), 9.83 (s, 1H, CONH); *m/z* 579.2 ($M^+ + 1$).

*5.1.2.2. (2-Morpholin-4-yl-ethyl)-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**4**).* Yellow solid (80 mg, 29% yield); R_f 0.50 (DCM/MeOH 85/15); mp = 46 °C; P_{HPLC} 95%, $t_R = 11.48$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.37–1.39 (m, 2H, CH_2), 1.52–1.56 (m, 4H, CH_2), 2.30–2.42 (m, 10H, CH_2), 2.44–2.47 (m, 4H, CH_2), 3.05 (s, 2H, $COCH_2N$), 3.47–3.49 (m, 2H, CH_2NHCO), 4.99 (s, 2H, CH_2Ar), 6.95 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.02 + 7.32 + 7.71 (s, 3H, Arom-H), 7.16 (t, $J = 5.5$ Hz, 1H, OCONH), 7.56 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.89 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.47 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.15 (s, 1H, NH), 9.75 (s, 1H, CONH); *m/z* 580.5 ($M^+ + 1$).

5.1.3. General procedure for the synthesis of compounds *5–8*

To a solution of 3-(diethylamino)propylamine or 1-(3-aminopropyl)-4-methylpiperazine (0.26 mmol, 1 eq.) in anhydrous DCM (5 mL) were added, at 0 °C, triphosgene (26 mg, 0.09 mmol, 0.33 eq.) and, after 5 min, NEt_3 (109 μL, 0.78 mmol, 3 eq.). After stirring the mixture for 10 min at room temperature and cooling to 0 °C, a solution of compound **1** (100 mg, 0.24 mmol, 0.9 eq.) in an anhydrous DCM/DMF 10/1 (5.5 mL) was added dropwise. After further stirring at room temperature overnight, the mixture was washed with 2 × 10 mL $NaHCO_3$ 1 M, the organic layer dried over $MgSO_4$, filtered and concentrated. The residue was purified by TLC (DCM/MeOH/NH_4OH 85/15/1) to yield the desired compound.

*5.1.3.1. (3-Diethylamino-propyl)-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**5**).* Yellow solid (45 mg, 33% yield); R_f 0.55 (DCM/MeOH/NH_4OH 85/15/1); P_{HPLC} 95%, $t_R = 14.38$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.87 (t, $J = 7.1$ Hz, 6H, $N(CH_2CH_3)_2$), 1.37–1.40 (m, 2H, CH_2), 1.45–1.55 (m, 6H, CH_2), 2.28–2.33 (m, 2H, CH_2), 2.36 (q, $J = 7.1$ Hz, 4H, $N(CH_2CH_3)_2$), 2.42–2.46 (m, 4H, CH_2), 2.99 (m, 2H, CH_2CONH), 3.05 (s, 2H, $COCH_2N$), 4.98 (s, 2H, CH_2Ar), 6.95 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.02 + 7.31 + 7.72 (s, 3H, Arom-H), 7.25 (t, $J = 5.5$ Hz, 1H, OCONH), 7.55 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.88 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.47 (d, $J = 5.3$ Hz, 1H, Quin-H_2, 9.15 (s, 1H, NH), 9.76 (s, 1H, CONH); ^{13}C NMR (DMSO-d_6) δ (ppm): 12.53 ($N(CH_2CH_3)_2$), 24.40 (CH_2), 26.34 (CH_2), 27.84 (CH_2), 47.07 ($N(CH_2CH_3)_2$), 50.70 (CH_2), 54.89 (CH_2), 63.52 ($COCH_2N$), 65.68 ($ArCH_2$), 103.07 (Quin-C_3), 113.27 + 114.84 + 117.08 (Arom-CH), 125.44 (Quin-C_5), 125.86 (Quin-C_6), 128.53 (Quin-C_8), 152.78 (Quin-C_2); *m/z* 580.3 ($M^+ + 1$).

*5.1.3.2. [3-(4-Methyl-piperazin-1-yl)-propyl]-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**6**).* Yellow solid (25 mg, 17% yield); R_f 0.75 (DCM/MeOH/NH_4OH 80/20/2); mp = 50 °C; P_{HPLC} 96%, $t_R = 13.51$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.37–1.39 (m, 2H, CH_2), 1.49–1.56 (m, 6H, CH_2), 2.11 (s, 3H, CH_3), 2.20–2.27 (m, 10H, CH_2), 2.43–2.45 (m, 4H, CH_2), 3.06 (s, 2H, NCH_2CO), 3.47–3.48 (m, 2H, CH_2NHCO), 4.98 (s, 2H, $ArCH_2$), 6.95 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.02 + 7.32 + 7.71 (s, 3H, Arom-H), 7.25 (t, $J = 5.7$ Hz, 1H, OCONH), 7.56 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.88 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.41 (d, $J = 9.1$ Hz, 1H,

Quin-H_5), 8.47 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.14 (s, 1H, NH), 9.76 (s, 1H, CONH); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.39 (CH_2), 26.32 + 27.47 (CH_2), 46.40 (CH_3), 53.33 + 55.46 (CH_2), 54.89 (CH_2), 63.50 (CO CH_2NH), 65.70 (Ar–CH_2), 103.07 (Quin-C_3), 113.26 + 114.82 + 117.06 (Arom-CH), 125.41 (Quin-C_5), 125.88 (Quin-C_6), 128.54 (Quin-C_8), 152.78 (Quin-C_2); *m/z* 607.4 ($M^+ + 1$).

*5.1.3.3. Benzyl-carbamic acid 3-(7-chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzyl ester (**7**).* To a solution of compound **1** (100 mg, 0.24 mmol, 1 eq.) in an anhydrous DCM/DMF 10/1 mixture (5.5 mL) was added, at 0 °C, benzylisocyanate (30 mg, 0.24 mmol, 1 eq.). After stirring at room temperature overnight, the reactive medium was concentrated and the residue purified by TLC (DCM/MeOH 85/15) to give compound **7** as a yellow solid (15 mg, 11% yield); R_f 0.75 (DCM/MeOH 85/15); P_{HPLC} 97%, $t_R = 19.21$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.37–1.39 (m, 2H, CH_2), 1.50–1.58 (m, 4H, CH_2), 2.42–2.46 (m, 4H, CH_2), 3.06 (s, 2H, $COCH_2N$), 4.19 (d, $J = 6.3$ Hz, 2H, $NHCH_2Ar$), 5.02 (s, 2H, $ArCH_2$), 6.96 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.05 + 7.35 + 7.72 (s, 3H, Arom-H), 7.18–7.32 (m, 5H, Arom-H), 7.56 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.84 (t, $J = 6.0$ Hz, 1H, OCONH), 7.89 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.3$ Hz, 1H, Quin-H_5), 8.45 (d, $J = 5.5$ Hz, 1H, Quin-H_2), 9.16 (s, 1H, NH), 9.77 (s, 1H, CONH); *m/z* 558.8 ($M^+ + 1$).

*5.1.3.4. 3-(7-Chloro-quinolin-4-ylamino)-5-(2-piperidin-1-yl-acetylamino)-benzoic acid (**8**).* To a solution of compound **1** (500 mg, 1.2 mmol, 1 eq.) in 30 mL of acetonitrile was added a solution of periodic acid (1.07 g, 4.7 mmol, 4 eq.) and catalytic amount of CrO_3 in 20 mL of acetonitrile. After stirring at room temperature for 2 h, the mixture was filtered washed with acetonitrile and concentrated. The residue was the purified by TLC (DCM/MeOH 8/2) to give compound **8** as a brown solid (450 mg, 90% yield); R_f 0.15 (DCM/MeOH 85/15); mp > 250 °C; P_{HPLC} 100%, $t_R = 12.14$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.40–1.70 m, 6H, CH_2), 2.20–2.70 (m, 4H, CH_2), 2.98 (s, 2H, $COCH_2N$), 7.05 (d, $J = 5.6$ Hz, 1H, Quin-H_3), 7.64 (m, 1H, Quin-H_6), 7.65 + 7.91 + 7.97 (s, 3H, Arom-H), 7.93 (d, $J = 3.6$ Hz, 1H, Quin-H_8), 8.40 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.54 (d, $J = 5.6$ Hz, 1H, Quin-H_2), 9.58 (s, 1H, NH), 10.66 (s, 1H, COOH); *m/z* 439.5 ($M^+ + 1$).

*5.1.4. General procedure for access to amides **9–18***

To a solution of compound **8** (150 mg, 0.34 mmol, 1 eq.) in a 5.5 mL of a DCM/DMF 10/1 mixture were added PyBroP (240 mg, 0.51 mmol 1.5 eq.), the desired amine (0.51 mmol, 1.5 eq.) and DIEA (60 μL, 0.34 mmol, 1 eq.). After stirring at room temperature overnight, the mixture was concentrated then the residue was purified by TLC (DCM/MeOH/NH_4OH 90/10/0 to 80/20/0.5) to give the desired compound.

*5.1.4.1. 3-(7-Chloro-quinolin-4-ylamino)-N-(2-dimethylamino-ethyl)-5-(2-piperidin-1-yl-acetylamino)-benzamide (**9**).* Yellow solid (20 mg, 11% yield); R_f 0.10 (DCM/MeOH 80/20); mp = 157 °C; P_{HPLC} 100%, $t_R = 11.35$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.55–1.75 (m, 6H, CH_2), 3.00 (m, 4H, CH_2), 2.82 (s, 6H, NMe_2), 3.13 (s, 2H, $COCH_2N$), 3.23 (m, 2H, CH_2), 3.57 (m, 2H, CH_2), 6.98 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 7.60 + 7.82 + 7.88 (s, 3H, Arom-H), 7.65 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 1H, Quin-H_6), 7.95 (d, $J = 2.0$ Hz, 1H, Quin-H_8), 8.46 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.54 (d, $J = 5.4$ Hz, 1H, Quin-H_2), 8.67 (t, $J = 5.8$ Hz, 1H, CONH), 9.50 (s, 1H, NH), 10.70 (s, 1H, CONH); *m/z* 509.6 ($M^+ + 1$).

*5.1.4.2. 3-(7-Chloro-quinolin-4-ylamino)-N-(3-dimethylamino-propyl)-5-(2-piperidin-1-yl-acetylamino)-benzamide (**10**).* Yellow solid (35 mg, 20% yield); R_f 0.10 (DCM/MeOH 80/20); mp = 164 °C; P_{HPLC} 100%, $t_R = 11.42$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.80–1.95 (m, 8H, CH_2), 2.65 (m, 4H, CH_2), 2.77 (s, 6H, NMe_2), 3.05–3.15 (m, 4H, CH_2), 4.19 (m, 2H, CH_2), 6.94 (d, $J = 5.5$ Hz, 1H, Quin-H_3), 7.71 + 7.94 + 7.99 (s, 3H, Arom-H), 7.84 (dd, $J = 9.0$ Hz, $J = 1.8$ Hz, 1H, Quin-H_6), 8.05 (d, $J = 2.1$ Hz, 1H, Quin-H_8), 8.61 (d, $J = 6.1$ Hz, 1H, Quin-H_2), 8.72 (d, $J = 9.0$ Hz, 1H, Quin-H_5), 8.74 (t, $J = 4.6$ Hz, 1H, CONH), 9.42 (s, 1H, NH), 11.00 (s, 1H, CONH); *m/z* 523.7 ($M^+ + 1$).

*5.1.4.3. 3-(7-Chloro-quinolin-4-ylamino)-N-(2-dimethylamino-ethyl)-5-(2-piperidin-1-yl-acetylamino)-N-methylbenzamide (**11**).* Yellow solid (20 mg, 12% yield); R_f 0.40 (DCM/MeOH 90/10); mp = 90 °C; P_{HPLC} 98%, $t_R = 10.88$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20–1.25 (m, 2H, CH_2), 1.55 (m, 4H, CH_2), 2.60–2.70 (m, 17H, CH_2 and CH_3), 2.97 (s, 2H, $COCH_2N$), 6.99 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.09 + 7.46 + 7.78 (s, 3H, Arom-H), 7.58 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.90 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.41 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.50 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.26 (s, 1H, NH), 10.04 (s, 1H, CONH); *m/z* 523.4 ($M^+ + 1$).

*5.1.4.4. 3-(7-Chloro-quinolin-4-ylamino)-N-(2-pyrrolidin-1-yl-ethyl)-5-(2-piperidin-1-yl-acetylamino)-benzamide (**12**).* Yellow solid (30 mg, 16% yield); R_f 0.35 (DCM/MeOH 80/20); mp = 88 °C; P_{HPLC} 100%, $t_R = 11.71$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.35–1.40 (m, 2H, CH_2), 1.50–1.55 (m, 4H, CH_2), 1.80–1.85 (m, 4H, CH_2), 2.85–3.00 (m, 10H, CH_2), 3.10 (s, 2H, $COCH_2N$), 3.10–3.15 (m, 2H, CH_2), 6.98 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.52 + 7.78 + 7.91 (s, 3H, Arom-H), 7.56 (dd, $J = 9.0$ Hz, $J = 2.3$ Hz, 1H, Quin-H_6), 7.89 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.0$ Hz, 1H, Quin-H_5), 8.49 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 8.58 (t, $J = 5.2$ Hz, 1H, CONH), 9.25 (s, 1H, NH), 9.90 (s, 1H, CONH); *m/z* 535.2 ($M^+ + 1$).

*5.1.4.5. 3-(7-Chloro-quinolin-4-ylamino)-N-(2-morpholin-1-yl-ethyl)-5-(2-piperidin-1-yl-acetylamino)-benzamide (**13**).* Yellow solid (30 mg, 16% yield); R_f 0.55 (DCM/MeOH 80/20); mp = 85 °C; P_{HPLC} 96%, $t_R = 11.28$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.40–1.45 (m, 2H, CH_2), 1.60 (m, 4H, CH_2), 2.45–2.50 (m, 4H, CH_2), 2.67 (s, 2H, $COCH_2N$), 3.12 (t,

$J = 7.2$ Hz, 2H, CH_2), 3.55–3.60 (m, 10H, CH_2), 6.98 (d, $J = 5.4$ Hz, 1H, Quin-H_3), 7.51 + 7.72 + 7.91 (s, 3H, Arom-H), 7.56 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.90 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.41 (t, $J = 9.6$ Hz, 1H, CONH), 8.42 (d, $J = 9.0$ Hz, 1H, Quin-H_5), 8.49 (d, $J = 5.4$ Hz, 1H, Quin-H_2), 9.29 (s, 1H, NH), 10.11 (s, 1H, CONH); *m/z* 550.5 (M^+).

*5.1.4.6. N-[3-(7-Chloro-quinolin-4-ylamino)-5-piperidin-1-yl-carbonyl-phenyl]-2-piperidin-1-yl-acetamide (**14**).* Yellow solid (105 mg, 61% yield); R_f 0.50 (DCM/MeOH 90/10); P_{HPLC} 100%, $t_R = 13.97$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20–1.25 (m, 4H, CH_2), 1.45–1.50 (m, 8H, CH_2), 2.45–2.50 (m, 8H, CH_2), 3.06 (s, 2H, $COCH_2N$), 7.01 + 7.36 + 7.76 (s, 3H, Arom-H), 7.03 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.60 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.92 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.52 (d, $J = 5.4$ Hz, 1H, Quin-H_2), 9.38 (s, 1H, NH), 10.43 (s, 1H, CONH); *m/z* 505.4 (M^+).

*5.1.4.7. N-[3-(7-Chloro-quinolin-4-ylamino)-5-morpholin-1-ylcarbonyl-phenyl]-2-piperidin-1-yl-acetamide (**15**).* Yellow solid (85 mg, 50% yield); R_f 0.45 (DCM/MeOH 90/10); P_{HPLC} 96%, $t_R = 12.19$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.40 (m, 2H, CH_2), 1.55–1.60 (m, 4H, CH_2), 2.45–2.55 (m, 4H, CH_2), 3.06 (s, 2H, $COCH_2N$), 3.10–3.60 (m, 8H, CH_2), 7.01 (d, $J = 5.2$ Hz, 1H, Quin-H_3), 7.03 + 7.40 + 7.83 (s, 3H, Arom-H), 7.58 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.91 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.40 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.52 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.22 (s, 1H, NH), 9.95 (s, 1H, CONH); *m/z* 508.2 ($M^+ + 1$).

*5.1.4.8. N-[3-(7-Chloro-quinolin-4-ylamino)-5-(N-methylpiperazin-1-ylcarbonyl)-phenyl]-2-piperidin-1-yl-acetamide (**16**).* Yellow solid (55 mg, 32% yield); R_f 0.30 (DCM/MeOH 90/10); P_{HPLC} 100%, $t_R = 10.40$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.40 (m, 2H, CH_2), 1.55–1.60 (m, 4H, CH_2), 2.22 (s, 3H, NCH_3), 2.35–2.55 (m, 8H, CH_2), 3.06 (s, 2H, $COCH_2N$), 3.50–3.55 (m, 4H, CH_2), 7.00 + 7.40 + 7.82 (s, 3H, Arom-H), 7.04 (d, $J = 5.3$ Hz, 1H, Quin-H_3), 7.58 (dd, $J = 9.1$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.91 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.40 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.52 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.22 (s, 1H, NH), 9.96 (s, 1H, CONH); *m/z* 521.3 ($M^+ + 1$).

*5.1.4.9. N-[3-(7-Chloro-quinolin-4-ylamino)-5-(N-phenylpiperazin-1-ylcarbonyl)-phenyl]-2-piperidin-1-yl-acetamide (**17**).* Yellow solid (150 mg, 75% yield); R_f 0.50 (DCM/MeOH 90/10); P_{HPLC} 95%, $t_R = 15.73$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20–1.25 (m, 2H, CH_2), 1.55–1.60 (m, 4H, CH_2), 2.40–2.50 (m, 8H, CH_2), 3.02 (s, 2H, $COCH_2N$), 6.81 (t, $J = 7.3$ Hz, 1H, Arom-H), 6.96 (d, $J = 7.9$ Hz, 2H, Arom-H), 7.07 (d, $J = 5.6$ Hz, 1H, Quin-H_3), 7.09 + 7.42 + 7.83 (s, 3H, Arom-H), 7.22 (dd, $J = 8.2$ Hz, $J = 7.4$ Hz, 2H, Arom-H), 7.60 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.92 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.42 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.54 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.31 (s, 1H, NH), 10.11 (s, 1H, CONH); *m/z* 582.7 (M^+).

*5.1.4.10. N-[3-(7-Chloro-quinolin-4-ylamino)-5-(N-phenylpiperazin-1-ylcarbonyl)-phenyl]-2-piperidin-1-yl-acetamide (**18**).* Yellow solid (135 mg, 67% yield); R_f 0.55 (DCM/MeOH 90/10); P_{HPLC} 94%, $t_R = 12.40$ min; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20–1.25 (m, 2H, CH_2), 1.55–1.60 (m, 4H, CH_2), 2.40–2.50 (m, 12H, CH_2), 3.05 (s, 2H, $COCH_2N$), 3.52 (s, 2H, CH_2), 7.02 (d, $J = 5.5$ Hz, 1H, Quin-H_3), 7.05 + 7.37 + 7.80 (s, 3H, Arom-H), 7.25–7.35 (m, 5H, Arom-H), 7.60 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, Quin-H_6), 7.92 (d, $J = 2.2$ Hz, 1H, Quin-H_8), 8.40 (d, $J = 9.1$ Hz, 1H, Quin-H_5), 8.52 (d, $J = 5.3$ Hz, 1H, Quin-H_2), 9.29 (s, 1H, NH), 10.16 (s, 1H, CONH); *m/z* 597.3 ($M^+ + 1$).

5.2. Biological evaluation

5.2.1. 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 [20]. In vitro antiplasmodial activity was determined using a modification of the semi-automated microdilution technique of Desjardins [21]. *P. falciparum* CQ-sensitive (THAI/Thailand) and CQ-resistant (FcB1R/Colombia and K1/Thailand) strains were used in sensitivity testing. Stock solutions of chloroquine diphosphate and test compounds were prepared in sterile distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and introduced to asynchronous parasite cultures (1% parasitaemia and 1% final hematocrit) on 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. After freezing and thawing, the cells were harvested from each well onto glass fiber filters and the dried filters were counted in a scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated into the parasite nucleic acids 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.

5.2.2. Cytotoxicity test upon MRC-5 cells

A human diploid embryonic lung cell line (MRC-5, BioWhittaker 72211D) was used to assess the cytotoxic effects towards host cells. MRC-5 cells were seeded at 5000 cells per well in 96-well plates. After 24 h, the cells were washed and two-fold dilutions of the drug were added in 200 μL standard culture medium (RPMI + 5% FCS). The final DMSO concentration in the culture remained below 0.5%. The cultures were incubated with several concentrations of compounds (between 32 μM and 1.6 μM) at 37 °C in 5% CO_2–95% air for 7 days. Untreated cultures were included as controls. The cytotoxicity was determined using the colorimetric MTT assay [22] and scored as a % reduction of absorption at 540 nm of treated cultures versus untreated control cultures.

5.2.3. In vivo drug assays on Plasmodium berghei

Antimalarial activity was determined in mice infected with *P. berghei* (ANKA 65 strain). Four-week-old female Swiss mice (CD-1, 20–25 g) was intraperitoneally infected with about 10^7 parasitized erythrocytes, collected from the blood of an acutely infected donor animal. At the same time, the animals (three per group) were orally treated with the test compound at 40 mg/kg (drug formulation in 100% DMSO) to not interfere with the intraperitoneal infection. The treatment was continued during the 4 following days by intraperitoneal route because the absorption is more extensive with less first-pass metabolism. Three infected DMSO-dosed mice were used as control animals, they generally die between 7 and 10 days following infection. Drug activity was evaluated by the reduction of parasites at day 4 and the prolongation of the mean survival time (excess MST) compared to that of controls.

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