DOI: 10.1002/ejoc.200700612

# Novel 4-Aminoquinolines through Microwave-Assisted $S_NAr$ Reactions: a Practical Route to Antimalarial Agents

**Sergio Melato,[a] Paolo Coghi,[a] Nicoletta Basilico,[b] Davide Prosperi,[a] and Diego Monti*[a]**

**Keywords:** Heterocycles / Aromatic substitution / Antimalarial agents / Microwave irradiation / Aminoquinolines

4-Aminoquinolines have recently been indicated to be an important class of chemotherapeutic agents for artemisinin-based antimalarial combination therapy. A rapid, cheap, possibly clean and scalable route to 4-aminoquinolines endowed with multiple diversity is therefore badly needed. Classically, they have been prepared by means of $S_NAr$ reactions, requiring hazardous or costly reagents and conditions and complex purification procedures. In this paper, microwave flash-heating chemistry is shown to allow the efficient conversion of the available 4,7-dichloroquinoline into a library of aminoquinolines in high yields and purities, with no need for further purification steps and requiring very short reaction times. Some of the compounds in this library were active against chloroquine-sensitive and chloroquine-resistant parasite strains.



## Introduction

Malaria nowadays remains one of the world's greatest public health problems, especially in the developing countries. Despite numerous efforts expended to reduce its mortality and morbidity,[1] malaria still threatens approximately 2 billion people worldwide, being responsible for almost two million deaths per year, mostly among young children in sub-Saharan Africa.[2] *P. falciparum*, the causative agent of the most invasive, malignant form of malaria, is a particularly resistant parasite, which is known to have high adaptability by mutation.[3] This mutability makes the development of resistance to chemotherapies quite likely. In this context, quinoline compounds such as chloroquine (CQ, **1**), quinine (**2**), piperaquine (**3**), amodiaquine (**4**) and primaquine (**5**) (Figure 1) were developed and used clinically for the treatment of malaria.[4] In particular, several 4-amino-7-chloroquinolines have been found to possess strong antimalarial activity.[5] Nevertheless, the emergence of resistance towards this class of compounds in several *P. falciparum* strains makes it urgent to develop simple, efficient (and if possible cheap) new routes to novel substituted 4-aminoquinolines with improved effectiveness against resistant strains.

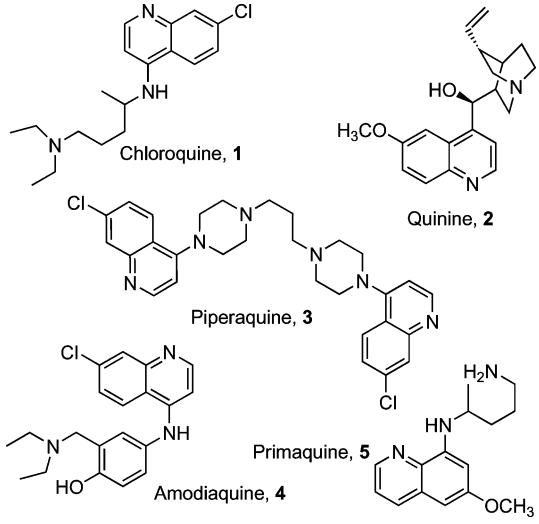



Figure 1. Quinoline-based antimalarial drugs.

One of the key issues in the attainment of a persistent antiplasmodial response is the careful identification of a suitable combination of antimalarial drugs.[6] Indeed, it is well documented that in many cases this approach results in a twofold effect: on one side, further development of resistance may be prevented, while on the other, the efficacy of the therapy could also be considerably improved. As a result, in order to prevent recrudescence, artemisinin derivatives, the most widely used antimalarial drugs since the discovery of the antiparasite properties of *Artemisia annua*, are frequently administered clinically in combination with

[a] Institute of Molecular Science and Technology, CNR,
Via G. Venezian 21, 20133 Milano, Italy
Fax: +39-0250313927
E-mail: diego.monti@istm.cnr.it
[b] Department of Public Health, Microbiology, Virology, University of Milan,
Via Pascal 36, 20133 Milano, Italy

longer-acting drugs.[7] Among these, 4-amino-7-chloroquinolines are considered promising partners of artemisinin derivatives for malaria combination therapy: examples include artesunate/amodiaquine and dihydroartemisinin/piperaquine (artekin) pairs.[8] As a consequence, there is an acute need for rapid and efficient synthetic routes to this important class of antimalarial compounds.

The 4-amino-7-chloroquinoline family may be directly accessed by selective displacement of the corresponding 4-halogen atom in the original heteroaryl halide backbone. Common methods thus involve $S_NAr$ reactions, which generally need elevated temperatures or catalysis by costly reagents, and the isolation of the products often requires complex, low-yielding purification steps.[9]

As an alternative to conventional heating, microwave irradiation has been demonstrated to be a clever strategy in various transformations, resulting in drastic increases in reaction rates and in substantial improvements of yields.[10] The growing interest in microwave-assisted reactions in organic synthesis resides in the fact that high yields and clean reactions may be obtained with the use of only small amounts of energy. Microwave heating of closed reaction vessels is a very energy efficient heating technique because only the reaction mixture is directly heated, thus minimizing wall effects and allowing superheating. Heating control is also highly reliable, since the energy input starts and stops as the power is turned on or off.[11] For this reason, much attention has been devoted to microwave-assisted organic synthesis in combinatorial medicinal chemistry.[12] However, there are only a few examples in the literature in which this methodology has been applied to antimalarial drug discovery. Microwave irradiation was exploited for the isolation of artemisinin,[13] and for the synthesis of quinoline derivatives by a Friedländer two-component condensation.[14] However, direct access to substituted 4-aminoquinolines from haloquinolines through $S_NAr$ reactions remains unexplored. Here we report the successful preparation of a small library of 4-amino-7-chloroquinolines obtained by aromatic nucleophilic substitution on the corresponding 4-chloro precursors under microwave irradiation conditions. This approach proved very valuable, allowing us to obtain the desired products in high yields, concomitantly decreasing the reaction times and the amounts of solvent required, and involving quick workups. Overall, this procedure appears to be a general strategy for the rapid, clean synthesis of libraries of aminoquinoline derivatives, and thus particularly suitable for combinatorial chemistry and high-throughput screening, and immediately adaptable to "green" scale-up.

## Results and Discussion

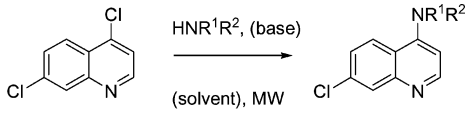
We examined the substitution reactions of 4,7-dichloroquinoline with different amines by heating mixtures in a microwave reactor at 6 bar pressure. Reaction parameters were adjusted depending on the characteristics of the nucleophilic amine. In general, dichloroquinoline (1 equiv.) and the amine (1.3 to 4.5 equiv.) were injected into an open microwave vial and, when appropriate, the solvent was added. The vessel was then hermetically sealed with a removable fitted cap and placed in the cavity of a microwave reactor fitted with a thermostat for temperature control. Heating was maintained for an appropriate period. In most cases, depending on the nucleophile reactivity, 1 equiv. base (*N*-methylmorpholine or solid NaOH) was also added before heating. To optimize temperature and reaction time, the course of the reaction was monitored by HPLC-MS analysis carried out on samples periodically taken from the reaction mixture. Isolation and purification of the products were accomplished by simple precipitation induced by addition of diethyl ether or 2 N NaOH (depending on the product solubility), followed by centrifugation and repeated washings of the solid matter with ethanolic ether or ethanolic aqueous solutions. In this way, laborious purification by multiphase extractions and time-consuming silica chromatography was unnecessary.

Our main goal was to design a general strategy to synthesize small 4-aminoquinolines as building blocks for novel antimalarial drugs. An inspection of the characteristics of the aminoquinoline library reported in Table 1 highlights the fact that three kinds of compounds were synthesized by use of three different classes of amines as nucleophiles. All the substituents used in our investigation are commercially available and cheap. To test our method, we first examined the reaction with known primary alkylamines (Entries 1–3 in Table 1).[15–17] In particular, we chose *N*-(3-aminopropyl)-imidazole (Entry 1) for three reasons: 1) the propyl chain is reminiscent of the recently discovered antiplasmodial compound AQ13,[18] 2) we used this amine to obtain a quinoline with a basic and buffering group at the end of the chain, useful for the proton trapping within the food vacuole, and 3) the imidazole group should stimulate interactions with the sulfate groups of the plasmodium membrane, thus increasing the cell uptake of the aminoquinoline compound. Since the heterocyclic compound piperaquine has recently been indicated to be a bis-quinoline antimalarial drug useful for combination therapy, in a second set of reactions we considered different piperazines in order to prepare quinoline-based piperaquine analogues (Entries 4–8). Finally, we evaluated five aromatic amines for the synthesis of heteroaromatic functionalized quinolines as amodiaquine analogues (Entries 9–13).

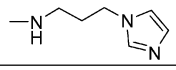
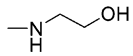
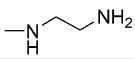
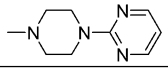
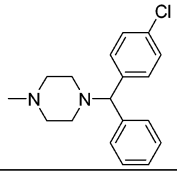
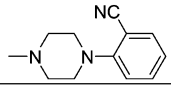
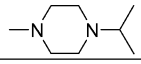
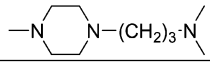
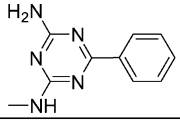
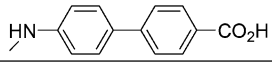
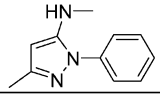
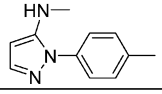
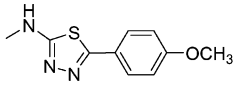
In general, the reactions were completed in 15–20 min, thus generating a library of thirteen different 4-amino-7-chloroquinolines in good to excellent yields. After various solvents, dichloroquinoline/nucleophile ratios, bases and thermal parameters had been surveyed, the best reaction conditions were set up depending on the use of primary, secondary or aromatic amines. Results and conditions are summarized in Table 1. Except in the cases of Entries 1–3, in which we found that solvent-free conditions were optimal, in all other cases dimethyl sulfoxide proved to be the best choice. Alternative organic solvents, including dimethyl formamide, acetonitrile, ethanol, tetrahydrofuran and water, were unsatisfactory, due to the formation of by-prod-

Table 1. Synthesis of 4-aminoquinoline library by microwave irradiation.




**6a-c** $R^1$ = H, $R^2$ = aliphatic
**7a-e** $R^1$, $R^2$ = *N*'-substituted piperazine
**8a-e** $R^1$ = H, $R^2$ = aromatic

| Entry | $NR^1R^2$ group | | Solvent | *T* [°C] | D/A[a] | Time [min] | Base[b] | % Yield |
|---|---|---|---|---|---|---|---|---|
| 1 |  | **6a** | – | 140 | 1/3.0 | 20 | – | 84 |
| 2 |  | **6b** | – | 140 | 1/4.0 | 22 | – | 92 |
| 3 |  | **6c** | – | 130 | 1/4.5 | 22 | – | 94 |
| 4 |  | **7a** | DMSO | 180 | 1/2.2 | 18 | NMM[c] | 83 |
| 5 |  | **7b** | DMSO | 180 | 1/2.2 | 18 | NMM[c] | 80 |
| 6 |  | **7c** | DMSO | 180 | 1/2.2 | 18 | NMM[c] | 87 |
| 7 |  | **7d** | DMSO | 180 | 1/2.2 | 18 | NMM[c] | 95 |
| 8 |  | **7e** | DMSO | 180 | 1/2.2 | 18 | NMM[c] | 92 |
| 9 |  | **8a** | DMSO | 130 | 1/1.5 | 12 | NaOH | 89 |
| 10 |  | **8b** | DMSO | 200 | 1/1.3 | 16 | NaOH | 85 |
| 11 |  | **8c** | DMSO | 200 | 1/1.8 | 16 | NaOH | 88 |
| 12 |  | **8d** | DMSO | 200 | 1/2.0 | 16 | NaOH | 85 |
| 13 |  | **8e** | DMSO | 200 | 1/1.3 | 18 | NaOH | 83 |

[a] 4,7-Dichloroquinoline/amine ratio. [b] 1 equiv. of base, when applicable. [c] NMM = *N*-methylmorpholine.

ucts deriving from solvent decomposition. Illustrative examples for each class of aminoquinoline derivatives are given in Table 2. As expected, only the chlorine substituent in the pyridine ring (4-position) is subject to nucleophilic displacement, while the chlorine in the phenyl ring is substantially inert.

Most of the chloroquinoline conversions required ca. 2 equiv. of amine to achieve optimal yields. By comparing our results under microwave-assisted conditions with the same transformations carried out with conventional heating, we found that in the first case the efficiency of the conversion was convincingly improved. Indeed, conventional

Table 2. Illustrative results for microwave-assisted $S_NAr$ reactions in different solvents.[a,b]

| Compound | Solvent | % Yield | Time [min] | $T$ [°C] |
|---|---|---|---|---|
| **6a** | EtOH | 61 | 20 | 140 |
| | acetonitrile | 72 | 20 | 140 |
| | DMF | 62 | 20 | 140 |
| | DMSO | 78 | 20 | 140 |
| **7e** | EtOH | 57 | 18 | 140 |
| | acetonitrile | 60 | 18 | 140 |
| | DMF | 66 | 18 | 180 |
| | DMSO | 92 | 18 | 180 |
| **8a** | EtOH | 41 | 15 | 130 |
| | acetonitrile | 32 | 15 | 130 |
| | DMF | 39 | 15 | 130 |
| | DMSO | 89 | 12 | 130 |

[a] One example was chosen from each class of aminoquinoline derivatives. [b] The use of THF and water as solvents led to decomposition of the starting material.

reaction environments always gave lower yields of the desired products. In particular, we observed good reactivities of substituted aromatic amines, which were completely unreactive under conventional heating conditions. In Table 3, the comparison between $S_NAr$ reactions carried out by microwave irradiation conditions and with conventional heating testifies to the considerable difference in terms of yields and reaction times.

Table 3. Comparison between $S_NAr$ reactions carried out under microwave irradiation conditions or with conventional heating.

| | Microwave irradiation | | | Conventional heating | | |
|---|---|---|---|---|---|---|
| | % Yield | Time [min] | $T$ [°C] | % Yield | Time [min] | $T$ [°C] |
| **6a** | 84 | 20 | 140 | 80 | 300 | 110 |
| **6b** | 92 | 22 | 140 | 92 | 240 | 140 |
| **6c** | 94 | 22 | 130 | 90 | 240 | 118 |
| **7a** | 83 | 18 | 180 | 78 | 360 | 130 |
| **7b** | 80 | 18 | 180 | 69 | 360 | 140 |
| **7c** | 87 | 18 | 180 | 66 | 360 | 135 |
| **7d** | 95 | 18 | 180 | 80 | 420 | 118 |
| **7e** | 92 | 18 | 180 | 70 | 360 | 120 |
| **8a** | 89 | 12 | 130 | 20 | 480 | 150 |
| **8b** | 85 | 16 | 200 | 0 | 480 | 189 |
| **8c** | 88 | 16 | 200 | 0 | 480 | 189 |
| **8d** | 85 | 16 | 200 | 0 | 480 | 189 |
| **8e** | 83 | 18 | 200 | 0 | 480 | 189 |

In order to assess the potential of this approach for high-throughput screening, all the compounds from the aminoquinoline library were screened to determine their in vitro antimalarial activity, by the pLDH assay, against D10 (CQ-sensitive) and W2 (CQ-resistant) strains of *P. falciparum*.[19] Some of the newly synthesized compounds, namely **6a**, **7d** and **7e**, exhibited good antimalarial activities ($IC_{50} < 1000$ nM) on both *P. falciparum* strains. The $IC_{50}$ values of these compounds and of CQ are reported in Table 4. According to WHO indications, any compound with an $IC_{50}$ value below a 300 nM threshold should be considered an active molecule for each corresponding strain test. Compound **6a** is more active against the CQ-sensitive strain than the CQ-resistant strain, while compounds **7d** and **7e** present similar activities on both stains. Interestingly, these compounds show improved activities, relative to CQ, on the W2 (CQ-R) strain. The other synthesized products were inactive ($IC_{50} > 1000$ nM) against both strains, and are not reported in Table 4.

Table 4. Antimalarial activities of newly synthesized 4-amino-7-chloroquinolines.[a,b]

| Compound | D10 CQ-S Strain $IC_{50}$ [nM] | W2 CQ-R Strain $IC_{50}$ [nM] |
|---|---|---|
| **6a** | 92 ± 20 | 290 ± 27 |
| **7d** | 257 ± 83 | 238 ± 53 |
| **7e** | 219 ± 43 | 242 ± 77 |
| CQ[c] | 30 ± 5 | 744 ± 28 |

[a] All synthesized compounds were screened. Those compounds not included in this Table showed $IC_{50}$ values >1000 nM against both D10 and W2 *P. falciparum* strains. [b] Data are the means ± SDs of three different experiments in duplicate. [c] CQ = chloroquine.

## Conclusions

In summary, a library of thirteen 4-aminoquinolines was synthesized as chloroquine analogues from commercially available 4,7-dichloroquinoline by means of microwave-assisted $S_NAr$ reactions. Of these products, three compounds showed significant antimalarial activities against plasmodia CQ-sensitive and CQ-resistant strains. All the compounds were obtained by a mild, clean and straightforward procedure, without formation of by-products. The use of microwave irradiation appears to be a promising, environmentally friendly and immediately scalable methodology through which to access novel, more effective antimalarial compounds for combination therapies.

## Experimental Section

**General Remarks:** All chemicals were of analytical grade and were used without further purification. Microwave-assisted reactions were performed with a Biotage Initiator™ (Uppsala, Sweden) high-frequency microwave synthesizer working at 2.45 GHz, fitted with magnetic stirrer and sample processor (30 vials capacity, 5 mL each); reaction vessels were Biotage microwave glass vials sealed with applicable cap; temperature was controlled through the internal IR sensor of the microwave apparatus. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker AVANCE 400 instrument. Mass spectra were acquired on a Bruker Esquire 3000 instrument (ionic trap), fitted with an Agilent 1100 analytical HPLC with a Waters Xterra column (4.6 × 50 mm, 3.5 μm granulometry) as stationary phase and an acetonitrile/water gradient as elution solvent (10 to 100% acetonitrile in 6 min, 10 μL injection). Elemental analyses were performed with a Perkin–Elmer Series II CHNS/O 2400 analyser.

**Typical Procedure (Entry 7):** 1-Isopropylpiperazine (795 μL, 5.54 mmol) and DMSO (2 mL) were placed in a 5 mL microwave reaction vessel equipped with a magnetic stirrer. After 10 min, 4,7-dichloroquinoline (500 mg, 2.52 mmol) and *N*-methylmorpholine (275 μL, 2.52 mmol) were added at room temperature. The reaction vessel was then placed in the cavity of the microwave reactor. The temperature was raised to 180 °C and the vessel was irradiated for

18 min at 6 bar pressure at the same temperature (the reaction temperature was modulated through the power switch and measured through the internal infrared sensor of the microwave apparatus). Samples of the reaction mixture were taken by syringe every 2 min, and were directly injected into the HPLC-MS analyzer. After complete disappearance of the MS signal corresponding to 4,7-dichloroquinoline, the reaction environment was cooled to room temperature, after which diethyl ether (5 mL) was added at 0 °C. Immediately after ether addition, a brown precipitate formed. The solid residue was isolated by centrifugation and the above liquid was discarded. The product was purified by washing several times with a diethyl ether/ethanol (9:1) mixture, to provide **7-chloro-4-(4-isopropylpiperazin-1-yl)quinoline** (**7d**) as a light brown solid (692 mg, 95% yield). The identity and purity of compound **7d** were checked by NMR spectroscopy and HPLC-MS. M.p. 155–157 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 1.05 (d, $J$ = 6.5 Hz, 6 H), 2.72 (m, 5 H), 3.16 (t, $J$ = 6.2 Hz, 2 H), 6.74 (d, $J$ = 5.3 Hz, 1 H), 7.32 (dd, $J$ = 8.8 and 2.0 Hz, 1 H), 7.87 (d, $J$ = 8.8 Hz, 1 H), 7.94 (d, $J$ = 2.0 Hz, 1 H), 8.61 (d, $J$ = 5.3 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 17.88, 48.80, 52.93, 53.67, 106.42, 121.24, 125.05, 125.65, 126.04, 133.57, 149.39, 152.07, 156.37 ppm. HPLC-MS: $m/z$ = 289.9 [M + 1]. $C_{16}H_{20}ClN_3$ (289.13): calcd. C 66.31, H 6.96, N 14.50; found C 66.22, H 7.01, N 14.58. HPLC purity > 99% (254 nm).

**7-Chloro-4-[3-(1*H*-imidazol-1-yl)propylamino]quinoline (6a):** Colourless solid, isolated yield 605 mg, 84%. M.p. 174–176 °C. $^1$H NMR (400 MHz, CD$_3$OD, TMS): $\delta$ = 2.23 (m, 2 H), 3.33 (t, $J$ = 6.9 Hz, 2 H), 4.19 (t, $J$ = 6.6 Hz, 2 H), 6.41 (d, $J$ = 4.9 Hz, 1 H), 7.00 (s, 1 H), 7.18 (s, 1 H), 7.37 (d, $J$ = 8.9 Hz, 1 H), 7.69 (s, 1 H), 7.77 (s, 1 H), 8.05 (d, $J$ = 8.9 Hz, 1 H), 8.33 (d, $J$ = 4.9 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, CD$_3$OD, TMS): $\delta$ = 29.17, 39.83, 44.53, 98.79, 117.61, 119.17, 122.62, 124.51, 126.07, 127.64, 134.85, 137.05, 148.34, 151.76, 151.79 ppm. HPLC-MS: $m/z$ = 287.0 [M + 1]. $C_{15}H_{15}ClN_4$ (286.10): calcd. C 62.83, H 5.27, N 19.54; found C 62.71, H 5.26, N 19.52. HPLC purity > 99% (254 nm).

**2-(7-Chloroquinolin-4-ylamino)ethanol (6b):** Colourless solid, isolated yield 515 mg, 92%. M.p. 213–215 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 3.33 (t, $J$ = 6.7 Hz, 2 H), 3.66 (t, $J$ = 6.7 Hz, 2 H), 4.86 (br s, 1 H), 6.48 (d, $J$ = 4.9 Hz, 1 H), 7.24 (m, 1 H), 7.42 (d, $J$ = 8.8 Hz, 1 H), 7.87 (s, 1 H), 8.25 (d, $J$ = 8.8 Hz, 1 H), 8.38 (d, $J$ = 6.7 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, CD$_3$OD, TMS): $\delta$ = 45.47, 59.59, 98.79, 118.23, 124.51, 127.96, 133.60, 149.28, 150.85, 152.73 ppm. HPLC-MS: $m/z$ = 222.8 [M + 1]. $C_{11}H_{11}ClN_2O$ (222.06): calcd. C 59.33, H 4.98, N 12.58; found C 59.28, H 4.96, N 12.62. HPLC purity > 99% (254 nm).

**4-[(2-Aminoethyl)amino]-7-chloroquinoline (6c):** Colourless solid, isolated yield 525 mg, 94%. M.p. 131–132 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 2.82 (t, $J$ = 6.7 Hz, 2 H), 3.25 (t, $J$ = 6.7 Hz, 2 H), 6.46 (d, $J$ = 5.0 Hz, 1 H), 7.24 (br s, 1 H), 7.42 (d, $J$ = 8.8 Hz, 1 H), 7.78 (s, 1 H), 8.27 (d, $J$ = 8.8 Hz, 1 H), 8.37 (d, $J$ = 5.0 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 40.76, 46.76, 98.91, 117.80, 124.39, 127.99, 133.68, 149.57, 150.77, 152.87 ppm. HPLC-MS: $m/z$ = 221.6 [M + 1]. $C_{11}H_{12}ClN_3$ (221.07): calcd. C 59.60, H 5.46, N 18.95; found C 59.78, H 5.52, N 18.86. HPLC purity > 99% (254 nm).

**7-Chloro-4-[4-(pyrimidin-2-yl)piperazin-1-yl]quinoline (7a):** Light brown solid, isolated yield 680 mg, 83%. M.p. 161–163 °C. $^1$H NMR (400 MHz, CDCl$_3$, TMS): $\delta$ = 3.19 (t, $J$ = 7.0 Hz, 4 H), 4.09 (t, $J$ = 7.0 Hz, 4 H), 6.47 (t, $J$ = 4.8 Hz, 1 H), 6.79 (d, $J$ = 5.2 Hz, 1 H), 7.38 (d, $J$ = 8.8 Hz, 1 H), 7.93 (d, $J$ = 8.8 Hz, 1 H), 8.07 (s, 1 H), 8.23 (d, $J$ = 4.8 Hz, 2 H), 8.64 (d, $J$ = 5.2 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, CDCl$_3$, TMS): $\delta$ = 43.75, 51.43, 109.16, 110.47, 121.96, 125.06, 126.41, 128.93, 135.06, 150.15, 151.90, 156.97, 157.80, 161.71 ppm. HPLC-MS: $m/z$ = 326.1 [M + 1]. $C_{17}H_{16}ClN_5$ (325.11): calcd. C 62.67, H 4.95, N 21.50; found C 62.85, H 4.96, N 21.43. HPLC purity > 99% (254 nm).

**7-Chloro-4-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}quinoline (7b):** Light brown solid, isolated yield 901 mg, 80%. M.p. 65–66 °C. $^1$H NMR (400 MHz, CDCl$_3$, TMS): $\delta$ = 3.49 (m, 4 H), 4.15 (m, 4 H), 5.49 (s, 1 H), 7.28 (d, $J$ = 5.8 Hz, 1 H), 7.40 (m, 5 H), 7.67 (d, $J$ = 8.8 Hz, 1 H), 7.75 (m, 4 H), 8.05 (s, 1 H), 8.16 (d, $J$ = 8.8 Hz, 1 H), 8.64 (d, $J$ = 5.8 Hz, 1 H) ppm. HPLC-MS: $m/z$ = 447.9 [M + 1]. $C_{26}H_{23}Cl_2N_3$ (447.13): calcd. C 69.64, H 5.17, N 9.37; found C 69.44, H 5.16, N 9.39. HPLC purity > 99% (254 nm).

**2-[4-(7-Chloroquinolin-4-yl)piperazin-1-yl]benzonitrile (7c):** Light brown solid, isolated yield 763 mg, 87%. M.p. 74–75 °C. $^1$H NMR (400 MHz, CDCl$_3$, TMS): $\delta$ = 3.38 (m, 4 H), 3.42 (m, 4 H), 6.83 (d, $J$ = 6.1 Hz, 1 H), 7.03 (m, 2 H) 7.36 (d, $J$ = 8.9 Hz, 1 H), 7.46 (m, 1 H), 7.53 (d, $J$ = 8.2 Hz, 1 H), 7.90 (d, $J$ = 8.9 Hz, 1 H), 7.99 (s, 1 H), 8.67 (s, 1 H) ppm. $^{13}$C NMR (100.6 MHz, CDCl$_3$, TMS): $\delta$ = 51.01, 52.30, 106.55, 109.35, 118.25, 118.97, 121.86, 122.50, 125.02, 126.40, 128.87, 133.96, 134.44, 135.12, 149.98, 151.82, 155.26, 156.78 ppm. HPLC-MS: $m/z$ = 349.1 [M + 1]. $C_{20}H_{17}ClN_4$ (348.11): calcd. C 68.52, H 4.72, N 16.39; found C 68.38, H 4.71, N 16.45. HPLC purity > 99% (254 nm).

**7-Chloro-4-{4-[3-(dimethylamino)propyl]piperazin-1-yl}quinoline (7e):** Light brown solid, isolated yield 770 mg, 92%. M.p. 149–151 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 2.13 (m, 2 H), 2.84 (s, 6 H), 3.19 (m, 2 H), 3.23 (m, 2 H), 3.53 (m, 4 H), 3.88 (m, 4 H), 7.30 (d, $J$ = 6.2 Hz, 1 H), 7.70 (dd, $J$ = 9.0 and 2.0 Hz, 1 H), 8.13 (d, $J$ = 2.0 Hz, 1 H), 8.19 (d, $J$ = 9.0 Hz, 1 H), 8.82 (d, $J$ = 6.2 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 19.22, 42.61, 51.02, 52.12, 54.10, 108.56, 119.47, 122.07, 122.81, 127.29, 128.14, 137.30, 143.51, 146.68, 158.65, 159.11, 159.41 ppm. HPLC-MS: $m/z$ = 333.1 [M + 1]. $C_{18}H_{25}ClN_4$ (332.18): calcd. C 64.95, H 7.57, N 16.83; found C 65.11, H 7.55, N 16.78. HPLC purity > 99% (254 nm).

**4-[(4-Amino-6-phenyl-1,3,5-triazin-2-yl)amino]-7-chloroquinoline (8a):** Pale yellow solid, isolated yield 780 mg, 89%. M.p. 202–204 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 7.50 (m, 4 H), 8.03 (s, 1 H), 8.31 (m, 3 H), 8.43 (d, $J$ = 8.9 Hz, 1 H), 8.83 (d, $J$ = 6.0 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 112.90, 120.19, 125.55, 125.99, 127.60, 127.95, 128.55, 131.74, 133.98, 136.25, 143.10, 149.12, 151.70, 165.39, 167.28, 170.72 ppm. HPLC-MS: $m/z$ = 349.0 [M + 1]. $C_{18}H_{13}ClN_6$ (348.09): calcd. C 61.98, H 3.76, N 20.09; found C 61.76, H 3.80, N 20.15. HPLC purity > 99% (254 nm).

**4′-[(7-Chloroquinolin-4-yl)amino]-1,1′-biphenyl-4-carboxylic Acid (8b):** Pale yellow solid, isolated yield 801 mg, 85%. M.p. 256–259 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 6.95 (d, $J$ = 5.7 Hz, 1 H), 7.41 (br s, 1 H) 7.58 (d, $J$ = 8.0 Hz, 1 H), 7.82 (d, $J$ = 8.0 Hz, 1 H), 7.89 (d, $J$ = 8.5 Hz, 1 H), 7.94 (d, $J$ = 7.9 Hz, 2 H), 7.99 (d, $J$ = 7.9 Hz, 2 H), 8.05 (s, 2 H), 8.57 (d, $J$ = 5.7 Hz, 1 H), 8.72 (d, $J$ = 8.5 Hz, 1 H) ppm. HPLC-MS: $m/z$ = 373.9. $C_{22}H_{15}ClN_2O_2$ (374.08): calcd. C 70.50, H 4.03, N 7.47; found C 70.38, H 4.04, N 7.52. HPLC purity > 99% (254 nm).

**7-Chloro-4-[(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)amino]quinoline (8c):** Yellow solid, isolated yield 741 mg, 88%. M.p. 206–208 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 2.28 (s, 3 H), 6.23 (s, 1 H), 6.38 (d, $J$ = 6.0 Hz, 1 H), 7.21 (m, 1 H), 7.33 (d, $J$ = 8.2 Hz, 2 H), 7.48 (d, $J$ = 8.7 Hz, 1 H), 7.58 (m, 2 H), 7.78 (s, 1 H), 8.21 (d, $J$ = 6.0 Hz, 1 H), 8.32 (d, $J$ = 8.2 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 13.99, 101.66, 118.57, 122.48,

123.37, 124.61, 124.85, 125.44, 126.33, 128.80, 134.27, 139.07, 140.69, 147.25, 148.28, 150.29 ppm. HPLC-MS: *m*/*z* = 335.1 [M + 1]. $C_{19}H_{15}ClN_4$ (334.10): calcd. C 68.16, H 4.52, N 16.73; found C 68.26, H 4.56, N 16.68. HPLC purity > 99% (254 nm).

**7-Chloro-4-[(3-methyl-1-methylphenyl-1*H*-pyrazol-5-yl)amino]quinoline (8d):** Yellow solid, isolated yield 716 mg, 85%. M.p. 254–255 °C. $^1$H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta$ = 2.25 (s, 3 H), 6.31 (br s, 1 H), 6.44 (br s, 1 H), 7.17 (m, 1 H), 7.46 (m, 3 H), 7.77 (m, 2 H), 8.26 (d, *J* = 6.2 Hz, 1 H), 8.32 (d, *J* = 8.8 Hz, 1 H) ppm. $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, TMS): $\delta$ = 20.42, 89.68, 101.58, 122.07, 122.82, 124.40, 125.09, 125.87, 128.13, 128.18, 128.75, 129.34, 129.46, 134.37, 136.48, 139.46, 139.99, 151.90 ppm. HPLC-MS: *m*/*z* = 335.0 [M + 1]. $C_{19}H_{15}ClN_4$ (334.10): calcd. C 68.16, H 4.52, N 16.73; found C 68.03, H 4.51, N 16.79. HPLC purity > 99% (254 nm).

**7-Chloro-4-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)amino]quinoline (8e):** Yellow solid, isolated yield 770 mg, 83%. M.p. 203–205 °C. $^1$H NMR (400 MHz, $CD_3OD$, TMS): $\delta$ = 3.89 (s, 3 H), 7.08 (m, 2 H), 7.85 (m, 3 H), 8.05 (s, 1 H), 8.65 (d, *J* = 8.7 Hz, 2 H), 8.80 (d, *J* = 6.3 Hz, 1 H) ppm. HPLC-MS: *m*/*z* = 369.0 [M + 1]. $C_{18}H_{13}ClN_4OS$ (368.05): calcd. C 58.61, H 3.55, N 15.19; found C 58.67, H 3.56, N 15.16. HPLC purity > 99% (254 nm).

**Parasite Growth:** *P. falciparum* cultures were carried out in vitro by Trager and Jensen's method with slight modifications.[20] The CQ-sensitive strain D10 and the CQ-resistant strain W2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone, Celbio) ($NaHCO_3$ 24 mM) medium with the addition of heat-inactivated A-positive human plasma (10%), Hepes (20 mM) and glutamine (2 mM). All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% ($O_2$), $CO_2$ (5%), $N_2$ (94%).

**Drug Susceptibility Assay on *P. falciparum*:** Compounds were dissolved in either water (chloroquine) or DMSO and were then diluted with a medium to achieve the required concentrations (final DMSO concentration < 1%, which is nontoxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR), and serial dilutions were made. Asynchronous cultures with parasitemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically ($OD_{650}$) by measuring the activity of the parasite lactate dehydrogenase (LDH), by a modified version of Makler's method in control and drug-treated cultures.[19b] Antimalarial activities are expressed as the 50% inhibitory concentrations ($IC_{50}$); each $IC_{50}$ value is the mean and standard deviation of at least three separate experiments performed in duplicate.

## Acknowledgments

We acknowledge D. Taramelli for helpful discussion. Financial support for this work has been provided by European Community, Antimal-LSHP-CT-2005-18834. S. M. is grateful to the University of Milan for a Ph.D. fellowship. We thank the Italian National Research Council (CNR) for financial support.

[1] World Health Organization **2006**: http://www.rollbackmalaria.org/malariaFAQ.html.
[2] a) S. I. Hay, C. A. Guerra, A. J. Tatem, A. M. Noor, R. W. Snow, *Lancet Infect. Dis.* **2004**, *4*, 327–336; b) A. K. Rowe, S. Y. Rowe, R. W. Snow, E. L. Korenromp, J. R. M. Armstrong Schellenberg, C. Stein, B. L. Nahlen, J. Bryce, R. E. Black, R. W. Steketee, *Int. J. Epidemiol.* **2006**, *35*, 691–704.
[3] a) C. A. Swales, P. L. Chiodini, B. A. J. Bannister, *Infect.* **2007**, *54*, 107–110; b) D. A. van Schalkwyk, T. J. Egan, *Drug Resist. Updates* **2006**, *9*, 211–226.
[4] M. Foley, L. Tilley, *Pharmacol. Ther.* **1998**, *79*, 55–87.
[5] R. Buller, M. L. Peterson, O. Almarsson, L. Leiserowitz, *Cryst. Growth Des.* **2002**, *2*, 553–562.
[6] a) S. Oyakhirome, M. Potschke, N. G. Schwarz, J. Dornemann, M. Laengin, C. O. Salazar, B. Lell, J. F. J. Kun, P. G. Kremsner, M. P. Grobusch, *Malar. J.* **2007**, *6*, 29–29; b) W. R. J. Taylor, J. Terlouw, P. L. Olliaro, N. J. White, P. Brasseur, F. O. ter Kuile, *Bull. World Health Org.* **2006**, *84*, 956–964.
[7] S. L. Nsobya, M. Joloba, C. Dokomajilar, G. Dorsey, P. J. Rosenthal, *Am. J. Trop. Med. Hyg.* **2006**, *75*, 110–110.
[8] A. R. Hasugian, H. L. E. Purba, E. Kenangalem, R. M. Wuwung, E. P. Ebsworth, R. Maristela, P. M. P. Penttinen, F. Laihad, N. M. Anstey, E. Tjitra, R. N. Price, *Clin. Infect. Dis.* **2007**, *44*, 1067–1074.
[9] B. J. Margolis, K. A. Long, D. L. T. Laird, J. C. Ruble, S. R. Pulley, *J. Org. Chem.* **2007**, *72*, 2232–2235, and references therein.
[10] a) C. O. Kappe, *Angew. Chem. Int. Ed.* **2004**, *43*, 6250–6284; b) P. Lidström, J. Tierney, B. Wathey, J. Westman, *Tetrahedron* **2001**, *57*, 9225–9283.
[11] M. Larhed, C. Moberg, A. Hallberg, *Acc. Chem. Res.* **2002**, *35*, 717–727.
[12] M. Larhed, A. Hallberg, *Drug Discov. Today* **2001**, *6*, 406–416.
[13] C.-Z. Liu, H.-Y. Zhou, Y. Zhao, *Anal. Chim. Acta* **2007**, *581*, 298–302.
[14] G. C. Muscia, M. Bollini, J. P. Carnevale, A. M. Bruno, S. E. Asís, *Tetrahedron Lett.* **2006**, *47*, 8811–8815.
[15] A. S. Tomcufcik, W. E. Meyer, J. W. Marsico, *Eur. Pat.* 446,604, **1991**, *SciFinder Scholar* AN **1992**:235628.
[16] I. Chiyanzu, C. Clarkson, P. J. Smith, J. Lehman, J. Gut, P. J. Rosenthal, K. Chibale, *Bioorg. Med. Chem.* **2005**, *13*, 3249–3261.
[17] a) C. C. Musonda, J. Gut, P. J. Rosenthal, V. Yardley, R. C. Carvalho de Souza, K. Chibale, *Bioorg. Med. Chem.* **2006**, *14*, 5605–5615; b) H. Zhang, V. R. Solomon, C. Hu, G. Ulibarri, *Biomed. Pharmacother.*; DOI: 10.1016/j.biopha.2007.04.007.
[18] V. R. Solomon, W. Haq, K. Srivastava, S. K. Puri, S. B. Katti, *J. Med. Chem.* **2007**, *50*, 394–398.
[19] a) P. J. Waako, B. Gumede, P. Smith, P. I. J. Folb, *Ethnopharmacol.* **2005**, *99*, 137–143; b) M. T. Makler, J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbins, D. J. Hinrichs, *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739–741.
[20] W. Trager, J. W. Jensen, *Science* **1976**, *193*, 673–675.

Received: July 4, 2007
Published Online: October 23, 2007