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Synthesis and *in vitro/in silico* evaluation of the antimalarial activity of potential amino-quinoline derivatives

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ABSTRACT

In this study, a series of *N*-arylamino-7-chloroquinolines were synthesized via an alkylation reaction involving aniline derivatives (**2**) and 4,7-dichloroquinoline (**1**). Additionally, the synthesis of a library of *N*-aryl-*N*-benzylamino-7-chloroquinoline analogues, modified on the aniline nitrogen, has also reported. A structure-activity relationship (SAR) study was conducted to evaluate the biological efficacy and safety of these derivatives against chloroquine-sensitive (*Pf*3D7) and chloroquine-resistant (*Pf*W2) *Plasmodium falciparum* strains. Compounds **5i** and **5c** showed promising efficacy against the *Pf*3D7 strain with IC₅₀ values of 0.25 μM and 0.54 μM, respectively, while compound **5l** demonstrated significant activity against the *Pf*W2 strain with an IC₅₀ of 5.82 μM. The cytotoxicity of these compounds was also evaluated on HUVEC cell lines. Additionally, their pharmacological and pharmacokinetic (ADME) properties were studied to predict their fate and identify promising candidates for further clinical studies.

1. Introduction

Malaria is a life-threatening parasitic disease affecting millions worldwide annually, primarily caused by *Plasmodium* parasites transmitted through the bites of infected female *Anopheles* mosquitoes [1]. According to the World Health Organization (WHO), there were approximately 249 million cases and 608,000 deaths due to malaria in 2022 [2], with the WHO African Region bearing the highest burden. In 2022, Africa accounted for 94 % (or 233 million) of all malaria cases and 95 % (or 580,000) of malaria-related deaths, with children under the age of five comprising nearly 78 % of these fatalities [2]. These figures highlight the urgency of the fight against this disease in the region. Prevention, treatment and awareness-raising are essential to ease the burden and protect the lives of the most vulnerable.

The traditional treatment for malaria, quinine, has lost efficacy due to adverse effects and increasing parasite resistance. Similarly, resistance to artemisinin-based combination therapies (ACTs), the current gold standard, is on the rise, posing a significant threat to global malaria control efforts [3–5]. This highlights the urgent need for new

therapeutic agents with novel mechanisms of action to combat multi-resistant *Plasmodium* strains [6].

Medicinal chemistry has been pivotal in the quest for new antimalarial drugs [7], with quinoline [8] derivatives emerging as a promising class of compounds [9]. These derivatives, characterized by their quinoline ring structure, exhibit potent antimalarial activity [8,10–12]. Today, several antimalarial and anticancer drugs incorporate quinoline-containing molecules due to their ability to inhibit parasite growth effectively [13–15].

Our research focuses on the rational design and synthesis of novel quinoline analogues with enhanced antimalarial activity and reduced side effects. We assessed the antimalarial activity of the quinoline [13, 16,17] derivatives synthesized using the *in vitro* model. We employed quantitative structure-activity relationship (SAR) studies to guide the selection of optimal molecular structures. Additionally, we conducted in-depth evaluations of the ADME (Absorption, Distribution, Metabolism, Excretion) properties of these compounds using *in silico* models (see in supplementary data). Understanding ADME properties is crucial for assessing the efficacy and safety of potential drug candidates [18,

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19].

In our pursuit of effective antimalarial agents against Pf3D7 and PfW2 strains, we identified amino-quinoline derivatives as promising candidates, with the general formula (A) (see Fig. 1). This study presents the synthesis, biological evaluation, and ADME profiling of these compounds, aiming to contribute to the development of safer, more effective, and accessible antimalarial therapies.

2. Materials and methods

2.1. Generality

^1H and ^{13}C NMR spectra were recorded at room temperature on a BRUKER UltraShield 300 spectrometer and tetramethylsilane (TMS) was used as internal reference. ^1H analyses were obtained at 300 MHz and ^{13}C analyses at 75 MHz. Chemical shifts δ are expressed in parts per million relatives to TMS ($\delta = 0.00$). Different deuterated solvents were used depending on the solubility of the products. For ^1H spectra, the abbreviations s, d, t, q, dd and m refer to signals in singlet, doublet, triplet, quadruplet, split doublet and multiplet form. Coupling constants J are expressed in Hertz (Hz). Mass spectroscopy analyses were performed on a Waters Micromass Quattro quadrupole/Q-TOF time-of-flight instrument equipped with ESI electrospray ionization or APCI atmospheric pressure chemical ionization.

Reactions were monitored by Macherey-Nagel Polygram Sil G/UV254 silica gel thin-layer chromatography (TLC), and products were detected under ultraviolet illumination at 254 nm. Product purification by column chromatography was carried out on silica gel (Merck Kieselgel 60).

2.2. Chemistry

2.2.1. General procedure for the synthesis of intermediates 3(a-d)

To a 10 mmol solution of 4,7-dichloroquinoline (1) dissolved in 20 ml absolute ethanol is added 1 equivalent of the corresponding amine (4-fluoroaniline, 3-fluoroaniline, 3,4-dichloroaniline and 3-chloro-2-methylaniline). The reaction mixture is stirred under refluxing ethanol for 4 h. After cooling to room temperature, the precipitate formed is filtered and washed several times with ethanol.

7-chloro-N-(4-fluorophenyl)quinolin-4-amine (3a). Yield: 87 %; MS: (ESI, +) for $\text{C}_{15}\text{H}_{10}\text{ClFN}_2$ [M+H] calculated 273.0517 m/z , found 273.0585 m/z ; ^1H NMR (300 MHz, CD_3OD) δ 11.18 (s, 1H), 8.90 (d, $J = 9.1$ Hz, 1H), 8.54 (d, $J = 7.0$ Hz, 1H), 8.25 (d, $J = 2.1$ Hz, 1H), 7.86 (dd, $J = 9.1, 2.1$ Hz, 1H), 7.58 (td, $J = 8.2, 6.5$ Hz, 2H), 7.48 – 7.33 (m, 2H), 6.83 (d, $J = 7.0$ Hz, 1H).

7-chloro-N-(3-fluorophenyl)quinolin-4-amine (3b). Yield: 85 %; MS: (ESI, +) for $\text{C}_{15}\text{H}_{10}\text{ClFN}_2$ [M+H] calculated 273.0517 m/z , found 273.0594 m/z ; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.36 (s, 1H), 8.97 (d, $J = 9.1$ Hz, 1H), 8.56 (d, $J = 7.0$ Hz, 1H), 8.24 (d, $J = 2.1$ Hz, 1H), 7.86 (dd, $J = 9.1, 2.1$ Hz, 1H), 7.61 (td, $J = 8.2, 6.5$ Hz, 1H), 7.48 – 7.33 (m, 2H), 7.27 (tdd, $J = 8.6, 2.6, 0.9$ Hz, 1H), 6.93 (d, $J = 7.0$ Hz, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 162.54 (d, $J = 245.4$ Hz, CF), 154.63 (CH), 143.59 (C^{IV}), 139.16 (C^{IV}), 138.97 (C^{IV}), 138.40, 131.54 (d, $J = 9.4$ Hz),

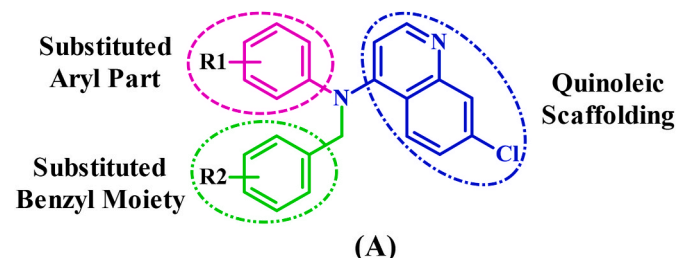


Fig. 1. General structure of the target molecule A.

127.40, 126.35, 121.26, 119.27, 116.15, 114.18 (d, $J = 20.8$ Hz), 112.46 (d, $J = 23.4$ Hz), 100.76.

7-chloro-N-(3,4-dichlorophenyl)quinolin-4-amine (3c). Yield: 89 %; MS: (ESI, +) for $\text{C}_{15}\text{H}_9\text{Cl}_3\text{N}_2$ [M+H] calculated 322.9831 m/z , found 322.9906 m/z ; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.21 (s, 1H), 8.86 (q, $J = 8.3, 6.5$ Hz, 1H), 8.59 (d, $J = 6.8$ Hz, 1H), 8.18 (q, $J = 2.3$ Hz, 1H), 7.94 – 7.72 (m, 3H), 7.52 (dd, $J = 8.6, 2.7$ Hz, 1H), 6.98 (d, $J = 6.8$ Hz, 1H).

^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 154.38 (CH), 143.80 (C^{IV}), 139.27 (C^{IV}), 138.36 (C^{IV}), 137.48 (C^{IV}), 131.99 (C^{IV}), 131.59 (CH), 129.34 (C^{IV}), 127.42 (CH), 126.98 (CH), 126.36 (CH), 125.38 (CH), 119.38 (CH), 116.25 (C^{IV}), 101.03 (CH).

7-chloro-N-(3-chloro-2-methylphenyl)quinolin-4-amine (3d). Yield: 95 %; MS: (ESI, +) for $\text{C}_{15}\text{H}_{10}\text{ClFN}_2$ [M+H] calculated 303.0378 m/z , found 303.0477 m/z ; ^1H NMR (300 MHz, $\text{Methanol}-d_4$) δ 7.05 (d, $J = 9.2$ Hz, 1H), 6.79 (dd, $J = 7.0, 1.2$ Hz, 1H), 6.44 (t, $J = 1.6$ Hz, 1H), 6.24 (dt, $J = 9.1, 1.7$ Hz, 1H), 5.99 (d, $J = 7.7$ Hz, 1H), 5.91 – 5.66 (m, 3H), 4.81 (s, 1H), 1.77 (s, 3H). ^{13}C NMR (75 MHz, $\text{Methanol}-d_4$) δ 156.27 (CH), 142.96 (C^{IV}), 140.00 (C^{IV}), 139.06 (C^{IV}), 136.43, 135.77, 133.88, 129.48, 128.04, 127.85, 126.05, 124.71, 119.13 (CH), 115.42 (CH), 100.08 (CH), 13.72 (CH_3).

2.2.2. General procedure for the synthesis of 5(a-d)

To a solution of compound 3a (0.37 mmol, 100 mg) in MeCN (20 mL) is slowly added 100 μL of DIPEA and 3 equivalents of a solution of alkylating agent (substituted brominated benzyl derivative) in MeCN (10 mL) under stirring. After 5–24 h of stirring at 100 °C, the reaction medium was cooled to room temperature and the precipitate formed was filtered and washed several times with petroleum ether.

N-(2-bromobenzyl)-7-chloro-N-(4-fluorophenyl)quinolin-4-amine (5a). Yield: 88 %; HRMS: (ESI, +) for $\text{C}_{22}\text{H}_{15}\text{BrClFN}_2$ [M+3H] calculated 443.0091 m/z , found 443.0168 m/z ; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.28 (s, 1H), 8.85 (d, $J = 8.9$ Hz, 1H), 8.62 (d, $J = 7.3$ Hz, 1H), 8.07 (d, $J = 2.1$ Hz, 1H), 7.98 (dd, $J = 9.1, 1.9$ Hz, 1H), 7.84 – 7.75 (m, 1H), 7.58 (dd, $J = 8.9, 4.8$ Hz, 2H), 7.52 – 7.40 (m, 2H), 7.38 – 7.29 (m, 2H), 6.85 (d, $J = 7.4$ Hz, 1H), 6.83 – 6.78 (m, 1H), 5.91 (s, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 161.15 (d, $J = 225.4$ Hz, CF), 155.37, 148.09, 139.65, 139.13, 133.46, 133.24, 130.38, 128.42, 128.07, 127.94, 127.83, 127.64, 126.71, 122.05, 117.94, 117.10, 116.79, 101.15, 57.06 (CH_2).

N-(3-bromobenzyl)-7-chloro-N-(4-fluorophenyl)quinolin-4-amine (5b). Yield: 31 %; HRMS: (ESI, +) for $\text{C}_{22}\text{H}_{15}\text{BrClFN}_2$ [M+3H] calculated 443.0091 m/z , found 443.0154 m/z ; ^1H NMR (300 MHz, CDCl_3) δ 8.76 (d, $J = 4.8$ Hz, 1H), 8.07 (d, $J = 2.2$ Hz, 1H), 7.64 (dd, $J = 9.1, 1.5$ Hz, 1H), 7.51 (d, $J = 1.9$ Hz, 1H), 7.46 – 7.35 (m, 1H), 7.34 – 7.22 (m, 2H), 7.17 (td, $J = 7.5, 1.5$ Hz, 1H), 7.12 – 7.01 (m, 1H), 7.01 – 6.72 (m, 4H), 5.07 (s, 2H).

N-(4-bromobenzyl)-7-chloro-N-(4-fluorophenyl)quinolin-4-amine (5c). Yield: 26 %; HRMS: (ESI, +) for $\text{C}_{22}\text{H}_{15}\text{BrClFN}_2$ [M+3H] calculated 443.0091 m/z , found 443.0166 m/z ; ^1H NMR (300 MHz, CDCl_3) δ 8.52 (d, $J = 8.7$ Hz, 1H), 7.51 (dd, $J = 8.8, 2.3$ Hz, 2H), 7.27 (d, $J = 3.1$ Hz, 1H), 7.23 (dd, $J = 8.6, 1.9$ Hz, 1H), 7.11 – 6.98 (m, 4H), 6.99 – 6.93 (m, 1H), 6.93 – 6.81 (m, 2H), 5.98 (d, $J = 8.0$ Hz, 1H), 5.02 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 139.70, 134.24, 132.44, 127.93, 127.74, 124.14, 122.31, 122.21, 115.99, 115.70, 114.72, 101.53, 55.04.

7-chloro-N-(2-fluorobenzyl)-N-(4-fluorophenyl)quinolin-4-amine (5d). Yield: 49 %; HRMS: (ESI, +) for $\text{C}_{22}\text{H}_{15}\text{ClF}_2\text{N}_2$ [M+H] calculated 381.0892 m/z , found 381.1014 m/z ; ^1H NMR (300 MHz, $\text{Methanol}-d_4$) δ 8.65 (d, 1H), 8.20 (d, $J = 2.0$ Hz, 1H), 7.85 (dd, $J = 9.1, 2.0$ Hz, 1H), 7.55 (dd, $J = 7.8, 5.5$ Hz, 2H), 7.45 (dd, $J = 7.3, 5.7$ Hz, 1H), 7.41 – 7.29 (m, 3H), 7.29 – 7.17 (m, 3H), 6.88 (d, 1H), 5.50 (s, 2H).

2.2.3. General procedure for synthesizing 5(f-j)

Typical Procedure: To a stirred solution of 7-chloro-N-(3,4-dichlorophenyl)quinolin-4-amine 3c (0.3 mmol, 1.0 equiv) in MeCN (20 mL) were added benzyl bromide derivative (0.40 mmol, 1.3 equiv) and i-

Pr₂EtN (DIPEA, 0.62 mmol, 2 equiv). The mixture was heated to 100 °C and stirred until the reaction was complete, after 7–48h, as monitored by TLC. The solution cooled to room temperature was filtered and the precipitate was washed several times with diethyl ether. Typical procedure: Spectroscopic data (¹H and ¹³C NMR) were in good agreement with those expected for the sample.

7-chloro-N-(2-chlorobenzyl)-N-(3,4-dichlorophenyl)quinolin-4-amine (5f). Yield: 73 %; HRMS: (ESI, +) for C₂₂H₁₄Cl₄N₂ [M+3H] calculated 448.9911 *m/z*, found 448.9959 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 9.0 Hz, 1H), 8.59 (d, *J* = 7.5 Hz, 1H), 8.02 (s, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 9.1 Hz, 2H), 7.71–7.56 (m, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 1H), 6.88 (d, *J* = 7.7 Hz, 1H), 5.93 (s, 2H).

7-chloro-N-(3-fluorobenzyl)-N-(3,4-dichlorophenyl)quinolin-4-amine (5g). Yield: 57 %; HRMS: (ESI, +) for C₂₂H₁₄Cl₂FN₂ [M+H] calculated 431.0207 *m/z*, found 431.0300 *m/z*; ¹H NMR (300 MHz, CDCl₃) δ 8.50 (d, *J* = 8.7 Hz, 1H), 7.45–7.31 (m, 2H), 7.31–7.20 (m, 2H), 7.11–6.94 (m, 4H), 6.89 (dt, *J* = 9.3, 2.2 Hz, 1H), 6.81 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.98 (d, *J* = 8.0 Hz, 1H), 5.09 (s, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 153.46 (d, *J* = 171.8 Hz), 140.26, 139.87, 137.71, 137.57, 132.73, 131.10, 130.99, 130.88, 128.00, 124.40, 123.09, 121.63, 121.59, 121.20, 115.57, 115.29, 114.87, 113.28, 112.98, 101.35, 55.23.

N-(3-bromobenzyl)-7-chloro-N-(3,4-dichlorophenyl)quinolin-4-amine (5h). Yield: 57 %; HRMS: (ESI, +) for C₂₂H₁₄BrCl₃N₂ [M+H] calculated 431.0207 *m/z*, found 431.0300 *m/z*; ¹H NMR (300 MHz, CDCl₃) δ 8.50 (d, *J* = 8.7 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.32–7.20 (m, 4H), 7.16–6.97 (m, 3H), 6.81 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.99 (d, *J* = 8.0 Hz, 1H), 5.07 (s, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 153.35, 152.30, 143.82, 142.18, 140.22, 137.60, 137.38, 132.75, 131.63, 130.88, 129.08, 128.62, 128.02, 124.60, 124.44, 123.50, 123.08, 121.19, 114.81, 101.40, 55.07.

N-(4-bromobenzyl)-7-chloro-N-(3,4-dichlorophenyl)quinolin-4-amine (5i). Yield: 90 %; HRMS: (ESI, +) for C₂₂H₁₄BrCl₃N₂ [M+3H] calculated 492.9406 *m/z*, found 492.9473 *m/z*; ¹H NMR (300 MHz, CDCl₃) δ 8.53 (d, *J* = 8.6 Hz, 1H), 7.55–7.45 (m, 2H), 7.39 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.30–7.17 (m, 3H), 7.07 (t, *J* = 4.3 Hz, 3H), 6.88 (d, *J* = 3.1 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 5.99 (d, *J* = 7.7 Hz, 1H₂), 4.54 (d, *J* = 2.5 Hz, 1H₁). ¹³C NMR (75 MHz, CDCl₃) δ 154.59, 140.27, 134.02, 132.73, 132.48, 132.12, 131.88, 130.87, 130.18, 128.40, 128.02, 127.75, 124.42, 123.11, 122.36, 121.22, 117.58, 114.89, 101.31, 55.21.

7-chloro-N-(4-nitrobenzyl)-N-(3,4-dichlorophenyl)quinolin-4-amine (5j). Yield: 82 %; HRMS: (ESI, +) for C₂₂H₁₄Cl₂N₃O₂ [M+H] calculated 458.0152 *m/z*, found 458.0336 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.82 (dd, *J* = 13.8, 8.3 Hz, 2H), 8.29–8.17 (m, 2H), 8.14 (d, *J* = 1.9 Hz, 1H), 7.92 (dd, *J* = 9.0, 1.9 Hz, 1H), 7.85 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.56 (d, *J* = 2.5 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 7.4 Hz, 1H), 6.12 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 155.03, 148.77, 147.27, 142.36, 139.85, 138.89, 137.19, 132.20, 131.85, 129.99, 127.82 (2CH), 127.20, 126.83, 125.69, 124.00 (2CH), 118.05, 117.52, 101.89, 56.15.

2.2.4. General procedure for synthesizing 5(k-n)

7-chloro-N-(3-fluorophenyl)quinolin-4-amine **3b** (0.37 mmol), *i*-Pr₂EtN (in excess) and benzyl bromide derivative (0.52 mmol, 1.4 equiv) were heated in refluxing acetonitrile (20 mL) for 48 h. After cooling to room temperature, the solution was diluted with aqueous sodium bicarbonate (NaHCO₃). The aqueous phase was extracted with dichloromethane (3 x 20 mL). The organic phases were washed with saturated NaCl (40 mL) and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The reaction product was purified on a silica gel chromatography column. Eluent: ethyl acetate/cyclohexane, 3/7.

4-((2-bromobenzyl)(3-fluorophenyl)amino)-7-chloroquinolinium (5k). Yield: 81 %; MS: (ESI, +) for C₂₂H₁₅BrClFN₂ [M+3H] calculated 443.0091 *m/z*, found 443.0172 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.73 (d, *J* = 9.0 Hz, 1H), 8.56 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.90 (d, *J* = 9.0 Hz, 1H), 7.82–7.71 (m, 1H), 7.62 (q,

J = 7.8 Hz, 1H), 7.47–7.18 (m, 5H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.89–6.74 (m, 1H), 5.85 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 154.93, 148.06, 139.08, 133.27, 133.23, 131.88, 131.71, 130.52, 128.46, 128.17, 127.67, 126.62, 122.00, 121.25, 117.82, 117.35, 114.40, 112.19, 101.45, 57.18.

N-(3-bromobenzyl)-7-chloro-N-(3-fluorophenyl)quinolin-4-amine (5l). Yield: 91 %; MS: (ESI, +) for C₂₂H₁₅BrClFN₂ [M+3H] calculated 443.0091 *m/z*, found 443.0167 *m/z*; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.43 (d, *J* = 8.7 Hz, 1H), 7.38 (dd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.30–7.10 (m, 4H), 7.02 (dd, *J* = 7.7, 1.8, 1.0 Hz, 1H), 6.95 (d, *J* = 1.9 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.72–6.52 (m, 3H), 5.91 (d, *J* = 8.0 Hz, 1H), 4.95 (s, 2H).

¹³C NMR (75 MHz, Chloroform-*d*) δ 163.84 (d, *J* = 245.4 Hz), 154.16, 139.93, 137.53, 137.42, 131.56, 130.85, 130.41, 130.29, 129.09, 128.04, 124.62, 124.28, 123.47, 117.03, 114.73, 109.17, 108.89, 108.44, 108.15, 101.66, 54.98.

7-chloro-N-(3-fluorobenzyl)-N-(3-fluorophenyl)quinolin-4-amine (5m). Yield: 85 %; MS: (ESI, +) for C₂₂H₁₅ClF₂N₂ [M+H] calculated 381.0892 *m/z*, found 381.0990 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.79 (dd, *J* = 8.4, 6.0 Hz, 1H), 8.16 (s, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.63 (q, *J* = 8.0 Hz, 1H), 7.49–7.34 (m, 4H), 7.29 (t, *J* = 8.5 Hz, 1H), 7.22–7.06 (m, 3H), 6.99 (d, *J* = 7.4 Hz, 1H), 5.94 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.05 (d, *J* = 20.25 Hz), 160.81 (d, *J* = 18.75 Hz), 154.89, 148.44, 139.58, 138.89, 137.58, 137.48, 131.72 (d, *J* = 9.2 Hz), 131.15 (d, *J* = 8.5 Hz), 127.61, 126.77, 122.74, 121.32, 118.08, 117.53, 115.23 (d, *J* = 20.9 Hz), 114.50 (d, *J* = 20.9 Hz), 113.73 (d, *J* = 22.4 Hz), 112.43 (d, *J* = 23.8 Hz), 101.46, 56.24.

7-chloro-N-(3-fluorophenyl)-N-(4-(trifluoromethyl)benzyl)quinolin-4-amine (5n). Yield: 83 %; MS: (ESI, +) for C₂₃H₁₅ClF₄N₂ [M+H] calculated 431.0860 *m/z*, found 431.0959 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.44 (d, *J* = 8.7 Hz, 1H), 7.75 (dd, *J* = 8.1, 3.9 Hz, 3H), 7.52 (d, *J* = 1.9 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.41–7.31 (m, 2H), 6.85 (td, *J* = 8.7, 2.6 Hz, 1H), 6.74 (td, *J* = 9.2, 8.5, 2.0 Hz, 2H), 6.03 (d, *J* = 7.9 Hz, 1H), 5.53 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.69, 153.61, 142.73, 141.32, 139.73, 136.42, 130.82, 130.69, 127.43, 127.14, 125.76, 125.71, 124.05, 117.57, 115.77, 109.08, 108.80, 108.35, 108.06, 100.46, 53.84.

2.2.5. Procédure générale de synthèse de 5(o-q)

A mixture of secondary amine **3b** (0.37 mmol) and K₂CO₃ (2.52 mmol) is dissolved in THF (20 mL). 30min later, after addition of 2 equivalents of the corresponding brominated benzyl (2-fluorobenzyl bromide, 4-bromobenzyl bromide), the mixture is kept stirring at 100 °C for 4–48h. The solvent is evaporated under reduced pressure. The resulting residue is dissolved in 20 mL of an aqueous solution of NaOH (10 %). The aqueous phase is extracted with dichloromethane (3x20). The organic phases are combined and dried over Na₂SO₄. Solvents are evaporated under reduced pressure. The product is purified on a silica chromatography column using a DCM/MeOH (100/10) mixture as eluent. The compounds are in the form of a yellow powder.

7-chloro-N-(3-fluorophenyl)-N-(4-nitrobenzyl)quinolin-4-amine (5o). Yield: 93 %; MS: (ESI, +) for C₂₂H₁₅ClFN₃O₂ [M+H] calculated 407.0837 *m/z*, found 408.0941 *m/z*; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.89 (dd, *J* = 11.0, 8.2 Hz, 1H), 8.24 (d, *J* = 2.1 Hz, 1H), 8.23–8.17 (m, 2H), 7.95 (dd, *J* = 9.0, 1.9 Hz, 1H), 7.66 (td, *J* = 8.1, 6.4 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.44 (m, 3H), 7.33 (td, *J* = 8.6, 2.6 Hz, 1H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.16 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.20, 155.04, 148.77, 147.33, 142.47, 139.82, 138.99, 131.81, 131.69, 127.90, 127.77, 127.00, 123.98, 121.50, 118.11, 117.47, 114.82, 114.54, 112.78, 112.47, 101.73, 56.04.

N-(4-bromobenzyl)-7-chloro-N-(3-fluorophenyl)quinolin-4-amine (5p). Yield: 66 %; MS: (ESI, +) for C₂₂H₁₄BrClFN₂ [M+3H] calculated 443.0091 *m/z*, found 443.0176 *m/z*; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.63 (d, *J* = 5.2 Hz, 1H), 8.08 (d, *J* = 2.0 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.56–7.44 (m, 1H), 7.43–7.27 (m, 1H), 7.11–6.99 (m, 6H), 6.99–6.84 (m, 1H), 5.99 (d, *J* = 8.1 Hz, 1H), 5.03 (s, 2H).

7-chloro-*N*-(2-fluorobenzyl)-*N*-(3-fluorophényl)quinolin-4-amine (5q). Yield: 100 %; MS: (ESI, +) for $C_{22}H_{15}ClF_2N_2$ [M+H] calculated 381.0892 *m/z*, found 381.1009 *m/z*; 1H NMR (300 MHz, DMSO- d_6) δ 8.83 (d, *J* = 9.1 Hz, 1H), 8.75 (d, *J* = 7.5 Hz, 1H), 8.27 (d, *J* = 1.9 Hz, 1H), 7.94 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.71 – 7.55 (m, 1H), 7.43 – 7.15 (m, 7H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.00 (s, 2H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 164.20, 154.86, 148.42, 139.60, 139.02, 131.74, 131.62, 130.83, 130.72, 129.16, 127.59, 126.82, 124.94, 121.35, 117.97, 117.39, 115.90 (d, *J* = 20.6 Hz), 115.19 (d, *J* = 86.5 Hz), 112.51 (d, *J* = 23.6 Hz), 101.41, 51.46.

2.2.6. General procedure for the synthesis of 5(*r-t*)

A mixture of the secondary amine **3d** (0.33 mmol) and 2.5 equivalents of a solution of the corresponding alkylating agent (2-bromobenzyl bromide, 3-bromobenzyl bromide, 4-bromobenzyl bromide) is dissolved in 20 mL of acetonitrile. After adding 100 μ L of excess DIPEA, the mixture is stirred at 100 °C for 5–7 h. The solution, cooled to room temperature, was filtered and the precipitate is rinsed with 25 mL of petroleum ether. After overnight suspension, the desired compounds are obtained as a white solid.

***N*-(2-bromobenzyl)-7-chloro-*N*-(3-chloro-2-methylphenyl)quinolin-4-amine (5r).** Yield: 83 %; MS: (ESI, +) for $C_{23}H_{17}BrCl_2N_2$ [M+3H] calculated 472.9952 *m/z*, found 473.0050 *m/z*; 1H NMR (300 MHz, DMSO- d_6) δ 8.86 (d, *J* = 9.0 Hz, 1H), 8.56 (d, *J* = 7.4 Hz, 1H), 8.02 (s, 1H), 7.96 (d, *J* = 8.9 Hz, 1H), 7.86 – 7.69 (m, 1H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.45 (q, *J* = 7.9 Hz, 2H), 7.33 (dd, *J* = 5.7, 3.5 Hz, 2H), 7.01 – 6.69 (m, 1H), 6.43 (d, *J* = 7.4 Hz, 1H), 5.88 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 155.57, 148.12, 139.65, 139.15, 134.96, 133.38, 133.26, 130.42, 129.33, 128.55, 128.44, 128.23, 127.68, 126.75, 126.36, 122.07, 117.97, 116.92, 101.24, 57.10(CH₂), 14.93(CH₃).

***N*-(3-bromobenzyl)-7-chloro-*N*-(3-chloro-2-methylphenyl)quinolin-4-amine (5s).** Yield: 92 %; MS: (ESI, +) for $C_{23}H_{17}BrCl_2N_2$ [M+3H] calculated 472.9952 *m/z*, found 473.0064 *m/z*; 1H NMR (300 MHz, DMSO- d_6) δ 8.72 (d, *J* = 9.0 Hz, 1H), 8.50 (d, *J* = 7.5 Hz, 1H), 8.07 (s, 1H), 7.80 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.56 – 7.47 (m, 3H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.34 – 7.26 (m, 2H), 7.20 (d, *J* = 7.7 Hz, 1H), 6.33 (d, *J* = 7.5 Hz, 1H), 5.78 (s, 2H), 2.24 (s, 3H).

***N*-(4-bromobenzyl)-7-chloro-*N*-(3-chloro-2-methylphenyl)quinolin-4-amine (5t).** Yield: 88 %; MS: (ESI, +) for $C_{23}H_{17}BrCl_2N_2$ [M+3H] calculated 472.9952 *m/z*, found 473.0038 *m/z*; 1H NMR (300 MHz, DMSO- d_6) δ 8.83 (d, *J* = 9.1 Hz, 1H), 8.73 (d, *J* = 7.4 Hz, 1H), 8.20 (d, *J* = 1.9 Hz, 1H), 7.92 (dd, *J* = 9.0, 1.9 Hz, 1H), 7.66 – 7.52 (m, 3H), 7.52 – 7.37 (m, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.46 (d, *J* = 7.3 Hz, 1H), 5.89 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 155.50, 134.08, 131.80 (2CH), 129.47, 128.91 (2CH), 128.39, 127.66, 126.56, 118.21, 100.95, 56.14 (CH₂), 14.67 (CH₃).

2.3. Biological procedure

2.3.1. In vitro test for growth and proliferation of *P. falciparum*

Compounds were tested against parasites of susceptible *P. falciparum* strain 3D7 (Pf3D7) and resistant *P. falciparum* W2 (PfW2), using the two-day fluorescence-based SYBR Green I approach [20]. Parasites were grown under standard conditions with minor modifications to 2.5 % hematocrit in RPMI 1640 medium with an initial parasitemia of 1 %. Compounds and negative control were prepared by double dilution, in the range 0.098–100 μ g/mL, in a 96-well flat-bottom plate to give a final volume in each well [21]. After 48 h incubation, plates were subjected to 3 freeze-thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in SYBR Green I lysis buffer. Plates were then incubated for a further hour at room temperature in the dark and examined for relative fluorescence units per well using epRealplex [22] Master. IC₅₀ values for active compounds were calculated by non-linear regression using ICEstimator Server version 1.2 [23].

2.3.2. In vitro cytotoxicity assay

HUVEC cells were cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum and 1 mM L-glutamine and incubated in 5 % CO₂ at 37 °C. Host cell cytotoxicity was assessed using the SYBR Green I assay as previously described. HUVEC cells were seeded in a 96-well plate at 100,000 cells/well and incubated for 24 h to adhere. After discarding the old medium, cells were incubated in medium containing eight concentrations (0.78–100 μ g/mL) of each extract in duplicate. After 48 h incubation, cells were visualized using an inverted microscope to check morphology or cell viability. The medium was then removed and replaced with SYBR Green I-free lysis buffer, and the plates subjected to 3 freeze-thaw cycles. The cell lysis suspension was diluted 1:2 in SYBR Green I lysis buffer. Incorporation of SYBR Green I into cellular DNA and IC₅₀ analysis were obtained as before.

3. Results and discussion

3.1. Chemistry

The synthetic route and chemical structures of the intermediate compounds studied are presented in Scheme 1. The preparation of *N*-arylamino-quinoline derivatives was carried out according to a known protocol [24,25], but in this synthesis we used optimized reaction conditions leading to compounds **3(a-d)** in higher yields (85–99 %). Accordingly, 4,7-dichloroquinoline **1** was treated with various substituted aromatic amines **2** in refluxing ethanol, to afford a series of four (4) *N*-arylamino-7-chloroquinoline derivatives **3(a-d)** in satisfactory yields.

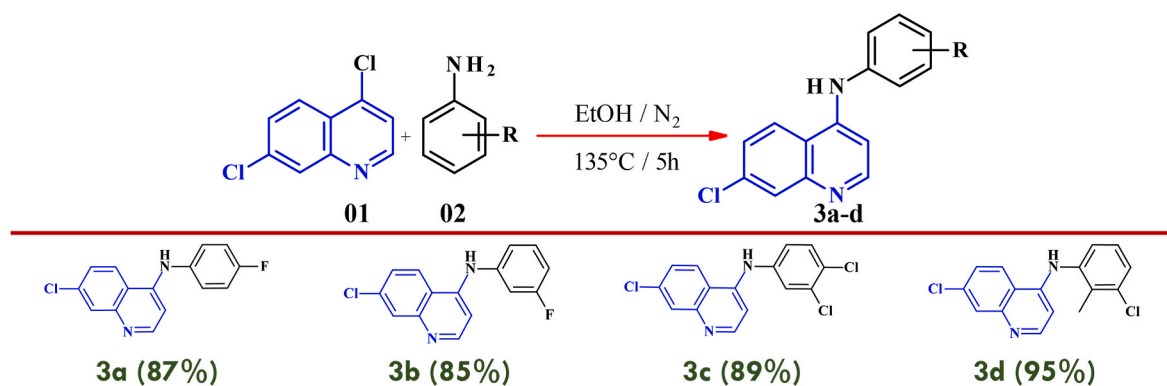
In the search for new ant-proliferative agents, the synthetic route used for the preparation of *N*-benzyl-*N*-arylamino-7-chloroquinoline molecules as quinoline analogues via the alkylation strategy is described in Scheme 2. The target analogues **5(a-t)** were prepared by a short synthetic route consisting of substituting the respective *N*-arylamino-7-chloroquinolines (**3a-d**), obtained previously, with various substituted benzyls (**4**) as described previously with slight modification [26]. In the presence of a small excess of diisopropylethylamine (DIPEA or Hünig base) or potassium carbonate K₂CO₃, we obtained excellent tertiary amine yields. We chose to study the direct benzylation of secondary *N*-arylamino-7-chloroquinoline using electron-poor benzyl bromides. Reactions were monitored by TLC and completed in 5–48 h in acetonitrile at 100 °C (see Table 1).

The advantage of this methodology lies in the introduction of the corresponding *N*-benzyl substituent in the second and final step. It should be remembered, however, that few reports have investigated the structural modifications made to the nitrogen in position 4, involving the double grafting of different alkyl or aryl substituents. The challenge in this synthesis is to synthesize tertiary amines from the 4-position nitrogen of amino-quinoline, with a view to making a systematic comparative study of secondary amines with the quinoline motif reported in the literature [27].

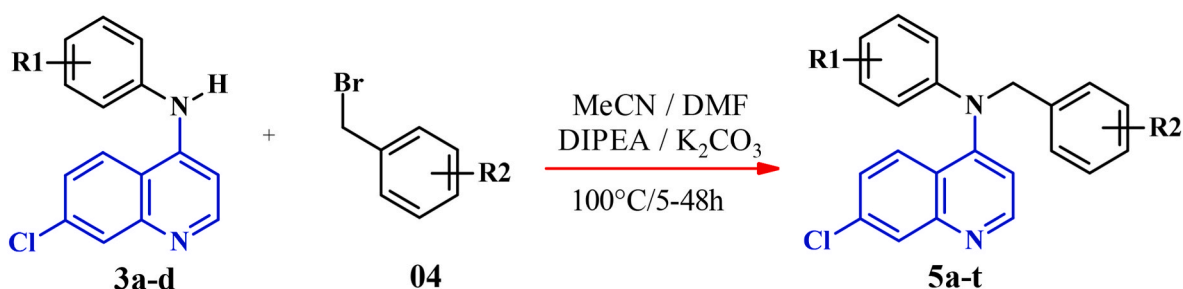
The final products were obtained smoothly as solids in medium to high yields. The *N*-benzyl-*N*-arylamino-7-chloroquinoline analogues and *N*-arylamino-7-chloroquinoline intermediates were fully characterized by nuclear magnetic resonance (NMR) and mass spectrometry (MS) analysis. Firstly, the main results of the analysis of the 1H NMR spectra of **5(a-t)** showed a singlet of approximately in the range 4.25–5.97 ppm linked to the methylenic hydrogens (CH₂) of the benzyl group, confirming the success of the substitution reaction. The same observation can be made in the ^{13}C NMR spectra, where a signal in the 45–60 ppm range is characteristic of the benzylic CH₂ carbon. All other signals expected for the final products were noted in the 1H and ^{13}C NMR spectra, and their identities were confirmed by MS analysis.

3.2. Biological activity of compounds

The resistance of *P. falciparum* to available drugs such as chloroquine

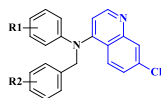


Scheme 1. Synthesis of 4-arylamino-7-chloroquinoline.



Scheme 2. Synthesis of N-aryl-N-benzylamino-7-chloroquinoline.

Table 1
Synthesis products of registered N-benzyl-N-arylamino-7-chloroquinoline analogues.



ID Compounds	R1	R2	Times (h)	Yields (%)
5a	F (p)	Br (o)	24	88
5b	F (p)	Br (m)	48	62
5c	F (p)	Br (p)	24	26
5d	F (p)	F (o)	5	49
5f	Cl (m), Cl (p)	Cl (o)	24	73
5g	Cl (m), Cl (p)	F (m)	7	57
5h	Cl (m), Cl (p)	Br (m)	24	57
5i	Cl (m), Cl (p)	Br (p)	20	90
5j	Cl (m), Cl (p)	NO ₂ (p)	12	82
5k	F (m)	Br (o)	24	84
5l	F (m)	Br (m)	24	91
5m	F (m)	F (o)	24	85
5n	F (m)	CF ₃ (p)	24	83
5o	F (m)	NO ₂ (p)	24	93
5p	F (m)	Br (p)	25	66
5q	F (m)	F (o)	25	100
5r	CH ₃ (o), Cl (m)	Br (o)	7	83
5s	CH ₃ (o), Cl (m)	Br (m)	5	92
5t	CH ₃ (o), Cl (m)	Br (p)	5	88

and artemisinin continues to grow, limiting therapeutic efficacy in the treatment of malaria [28]. In view of the above, there is an urgent need to design new pharmacologically active molecules [29].

A recent study on amino-quinolines clearly suggested that antimalarial activity, in particular the inhibition of β -hematin formation and drug accumulation at the target site, resides in the 4-aminoquinoline core [30]. Quinoline derivatives act by blocking the growth of heme crystals, a substance produced by the parasite responsible for malaria.

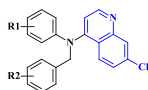
These molecules are easily obtained synthetically, and present an economically viable strategy for developing new molecule libraries. A set of compound validation criteria is described in the development of new antimalarial drugs. Compounds to be tested *in vitro* should preferably meet specific requirements: lethal drug concentration that reduces parasite viability by 50 % (IC₅₀) <1 μ M; minimum selectivity index (SI) values of 10, ideally above 100 [31,32]. The cytotoxicity test analyzes the survival rate of the cell line in the presence of the molecules. This makes it possible to assess the toxic effects of compounds on living cells. *In vitro* tests were carried out to assess the antiplasmodial activity of quinoline analogues against chloroquine-sensitive Pf3D7 and chloroquine-resistant PfW2 strains. Chloroquine was used as a positive control and as a reference, and the results of the biological tests are reported in Table 2.

The compounds tested were evaluated for their antimalarial profiles against CQ-susceptible Pf3D7 and CQ-resistant W2 strains of *P. falciparum*. Analysis of Table 2 revealed that these compounds were not as active as the standard drug, chloroquine (CQ), although compounds 3d (IC₅₀ = 1.61 μ M), 5c (IC₅₀ = 0.54 μ M), 5i (IC₅₀ = 0.25 μ M), 5j (IC₅₀ = 2.96 μ M), 5p (IC₅₀ = 3.25 μ M) and 5t (IC₅₀ = 2.20 μ M) showed good antimalarial activity among the compounds tested. These compounds showed a modest lethal dose (cytotoxicity) against the HUVEC cell line, comparable to that of chloroquine [33], on the other hand, these compounds are identified as high selectivity compounds. Based on the SAR study, we find that the alkyl in ortho-position on the N-aryl ring induced good antimalarial efficacy. This hypothesis could be confirmed when the (inactive) analogue 3c was converted into its 3d homologue (IC₅₀(3D7) = 1.61 μ M and IC₅₀(W2) = 4.48 μ M) with good cytotoxicity (CC₅₀ = 66.99 μ M) conferring it a higher selectivity than that of the reference molecule against the PfW2 strain.

Based on the observed results of the antiplasmodial activity of the molecules tested as quinoline analogues containing both aryl and benzyl moieties, the structure-activity relationship (SAR) can be summarized by indicating the change of the halogen atom in different positions on the N-benzyl ring which affects the antiplasmodial activity.

Table 2

In vitro antimalarial activity, cytotoxicity and selective index of quinoline derivatives.



Cpds ID	R1	R2	Antiplasmodial Activity in vitro: IC ₅₀ (μM ± SD)		CC ₅₀ (μM ± SD)	Selectivity Index: CC ₅₀ /IC ₅₀		
			Pf3D7	PfW2		Pf3D7	PfW2	
					3a			4-F
3b	3-F	–	>100	>100	55.22 ± 0.61	0.55	0.55	
3c	3,4-Cl	–	60.21 ± 3.20	>100	53.47 ± 0.32	0.89	0.53	
3d	2-CH ₃ , 3-Cl	–	1.61 ± 0.21	4.48 ± 1.54	66.99 ± 0.84	41.61	14.95	
End products								
5a	4-F	2-Br	>100	>100	86.87 ± 0.75	0.87	0.87	
5b	4-F	3-Br	23.19 ± 2.78	8.83 ± 2.06	59.54 ± 0.32	2.57	6.74	
5c	4-F	4-Br	0.54 ± 0.24	26.66 ± 2.49	49.53 ± 0.17	91.72	1.86	
5d	4-F	2-F	>100	>100	>100	1	1	
5f	3,4-Cl	2-Cl	19.87 ± 2.23	25.72 ± 2.02	78.14 ± 0.48	3.93	3.04	
5g	3,4-Cl	3-F	10.76 ± 0.14	8.54 ± 3.26	53.74 ± 0.28	4.99	6.29	
5h	3,4-Cl	3-Br	13.06 ± 1.89	13.48 ± 2.05	82.17 ± 0.60	6.29	6.10	
5i	3,4-Cl	4-Br	0.25 ± 0.10	25.85 ± 4.21	43.21 ± 0.12	172.84	1.71	
5j	3,4-Cl	4-NO ₂	2.96 ± 0.40	10.16 ± 2.51	77.56 ± 0.64	26.20	7.63	
5k	3-F	2-Br	71.17 ± 7.23	>100	82.42 ± 0.88	1.16	0.82	
5l	3-F	3-Br	12.50 ± 3.70	5.82 ± 1.35	64.54 ± 0.48	5.16	11.09	
5m	3-F	3-F	>100	>100	>100	1	1	
5n	3-F	4-CF ₃	44.97 ± 1.84	>100	61.24 ± 0.55	1.36	0.61	
5o	3-F	4-NO ₂	6.07 ± 2.74	12.22 ± 1.44	59.67 ± 0.28	9.83	4.88	
5p	3-F	4-Br	3.25 ± 0.44	58.25 ± 3.97	51.22 ± 0.60	15.76	0.88	
5q	3-F	2-F	>100	>100	>100	1	1	
5r	2-CH ₃ , 3-Cl	2-Br	5.99 ± 0.94	>100	71.63 ± 0.54	11.96	0.72	
5s	2-CH ₃ , 3-Cl	3-Br	0.91 ± 0.95	52.79 ± 7.06	75.98 ± 0.49	16.13	1.44	
5t	2-CH ₃ , 3-Cl	4-Br	2.20 ± 0.32	75.24 ± 4.89	66.28 ± 0.87	30.13	0.88	
CQ	/	/	0.019 ± 0.002	0.21 ± 0.002	>1	52.63	4.76	

IC₅₀ = Inhibitory Concentration at 50 %; CC₅₀ = cytotoxic concentration at 50 %; SI = Selectivity Index.

Initially, we explored the effect that *N*-benzyl on the amine in position 4 had on parasite destruction when the aryl ring was present on the amino-quinoline nitrogen. It was thus discovered that, of all the *N*-benzyl-*N*-arylamino-7-chloroquinoline analogues, the brominated analogues showed maximum ant-proliferative efficacy against malaria parasite strains, compared with the fluorinated and/or chlorinated analogues.

Looking specifically at the brominated analogues on the benzyl backbone, **5c** (0.54 μM) and **5i** (0.25 μM) having a bromine atom in the benzyl moiety in the para position, showed the best antplasmodial activity against the *Pf3D7* strain in an *in vitro* assay (Table 2). This

translates into strong inhibitory activity against *P. falciparum* parasite cells. Compounds **5c** and **5i** with CC₅₀ values of 49.53 μM and 43.21 μM respectively against the HUVEC cell line (cytotoxicity) also possess higher selectivity indices of 91.72 (3D7) and 172.84 (3D7) respectively. Lower activity was observed against *Pf3D7* strains when replacing the halogen with the NO₂ functional group **5j** (IC₅₀ = 2.96 μM) and **5o** (IC₅₀ = 6.07 μM). The analogue **5n**, with the CF₃ (trifluoromethyl) group in the para position of the benzyl ring, proved inactive against the resistant strain (IC₅₀ > 100) and ineffective against the susceptible strain (IC₅₀ of 44.97 μM). The SAR clearly suggest the crucial role of the nature and position of the substituent on the benzyl ring in antiplasmodial activity, and that these analogues are likely to be endowed with new leads that could overcome drug resistance. In general, having substituents at the para position on the *N*-benzyl skeleton resulted in a significant increase in antiplasmodial activity.

We then examined the effect of substituents in the meta position of the *N*-benzyl ring of the aminoquinoline scaffold. Among the *in vitro* results, analogues **3d**, **5b**, **5g** and **5l** showed the best IC₅₀ results with values of 4.48 μM, 8.83 μM, 8.54 μM and 5.82 μM respectively against the resistant strain (W2). Compounds **3d** (4.48 μM against W2) and **5l** (5.82 μM against W2), with CC₅₀ values of 66.99 μM and 64.54 μM respectively, had the highest selectivity index (14.95 and 11.09 respectively) above that of the reference molecule CQ.

All *N*-benzyl-*N*-arylamino-7-chloroquinoline analogues having a halogen in ortho-position on the *N*-benzyl backbone with an alkyl and/or halogen group in different positions on the *N*-aryl backbone were ineffective in inhibiting parasite growth. In contrast, the most active compounds (CC₅₀ ≥ 80 μM) against the HUVEC cell line were inactive against *P. falciparum*. No correlation was observed between antiplasmodial and anti-HUVEC activities. For example, analogues **5c** and **5i** show potent antiparasitic activity, but modest cytotoxicity, with CC₅₀ values < 50 μM. Whereas compounds **5a**, **5d** and **5m**, all inactive (IC₅₀ > 100), showed cytotoxicity in excess of 100 μM.

Compounds containing a para-positional substituent on the benzyl ring were the most active compounds, displaying moderate cytotoxic activity and excellent selectivity index. Among the *in vitro* results, analogues **5c** and **5i** were the most active, displaying the lowest IC₅₀ values of 0.54 μM and 0.25 μM respectively. Moreover, they also demonstrated higher selectivity indices, measuring 91.72 and 172.66 respectively. These results corroborate the criteria for the development of new anti-malarial compounds announced in the literature [32].

4. Conclusion

This multidisciplinary study combined the synthesis of nineteen (19) substituted *N*-benzyl-*N*-arylamino-7-chloroquinoline analogues with *in vitro* and *in silico* evaluation of their ADME properties. These analogues were tested for antimalarial activity and cytotoxicity. Among them, compounds **5c** (0.54 μM), and **5i** (0.25 μM) stood out for their highest *in vitro* antiplasmodial activity and their high selectivity index against the *Pf3D7* strain. In contrast, analogues **5b** (8.83 μM), **5g** (8.54 μM) and **5l** (5.82 μM) showed higher antimalarial activity against the *PfW2* strain.

We found that substituents in the para position on the benzyl ring, such as compounds **5c** and **5i**, significantly modulated the antiplasmodial activity of the molecules tested.

These molecules thus show very promising activity in the treatment of parasites, reinforcing their potential as effective drugs. The results obtained in this study could thus contribute to the development of new antimalarial drugs, which would be more effective, safer and targeted.

CRedit authorship contribution statement

Moussa Touré: Writing – original draft, Methodology, Investigation, Conceptualization. **Abdoulaye Gassama:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Oumar Sambou:**

Supervision, Formal analysis. **Christian Cavé**: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Sandrine Cojean**: Writing – review & editing, Validation, Supervision, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmc.2024.100241>.

Data availability

No data was used for the research described in the article.

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