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2,3,8-Trisubstituted Quinolines with Antimalarial Activity

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ABSTRACT

Combination therapy drugs are considered a fundamental way to control malaria as it mimimizes the risk of emergence of resistance to the individual partner drugs. Consequently, this type of therapy constitutes a driving force for the discovery of new drugs with different modes of action, since this will provide options for combining different drugs to achieve the optimum antimalarial treatment. In this context, a 2,3,8-trisubstitued quinoline compound was found in a high throughput screen (HTS) to show an excellent inhibition of *P. falciparum* NF54 (IC_{50} = 22 nM) and low cytotoxicity. We performed a detailed evaluation of the substituents to improve the metabolic stability and solubility liabilities of the original hit and identified derivatives with enhanced physicochemical and/or PK properties and that maintained biological activity. However the high potency was not retained on testing against drug resistant plasmodium strains.

Key words: antimalarial activity, trisubstituted quinoline, SAR, resistance.

INTRODUCTION

Malaria is an endemic human disease caused by the parasite *plasmodium* which uses the female *Anopheles* mosquito as a vector for transmition. It was estimated that 212 million new cases of malaria appeared around the world in 2015 (WHO 2016). Although malaria can be prevented and even cured, children under 5 still represent an extremely vulnerable group which is susceptible to infection and death. In addition, resistance to artemisin-based combination therapies (WHO 2016), the best available treatment nowadays, continues to develop, requiring the discovery of new medicines to treat this life threatening infection. Despite these issues, global efforts between public and private sectors, academia and NGO's (non-governamental organization) have been able to reduce the number of deaths and endemic regions. Because of that, since 2000, a 20% decline in the number of infected people and a 50% decline in deaths were observed along with 16% retraction of areas where this disease is present (WHO 2016).

From a historical point of view, the first reported example of an antimalarial agent was described in the 17th century, in the powder of a tree bark found in South America which, acording to folklore, was named cinchona after being used to treat the

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* Contribution to the centenary of the Brazilian Academy of Sciences.

Countess of Chinchon, Ana de Osorio in 1630 (Foley and Tilley 1998). Even after understanding the benefits of the cinchona tree, it was still two centuries later in 1820, that the French researchers Caventou and Pelletier were able to isolate the alkaloid quinine (**1**) in its pure form, which was shown to be responsible for the theraupeutic properties of the crude tree bark (Delepine 1951). Moreover, the chemical connectivity of this quinoline-containing compound started to be elucidated more deeply by the German chemist Paul Rabe in 1907 (Rabe 1907).

During World War II, demand for quinine (**1**) increased substantially, mainly after Java, a big supplier of quinine, fell under the control of the Japanese army and exports were stopped. In addition, synthetic efforts to prepare quinine (**1**), described initially by Rabe (Rabe and Kindler 1918), followed by Proštenik (Proštenik and Prelog 1943) and finally by Woodward (Woodward and Doering 1944, 1945), which was claimed to be the first formal synthesis (Smith and Williams 2008, Seeman 2007, Kaufman and Rúveda 2005, Stork et al. 2001) were not economically viable. As a result, pressure to develop alternative drugs with antimalarial activity increased. Chemical companies located in Germany and US were key players in the 1940's in the process to find synthetic compounds to treat malaria. Basically, all the compounds developed to kill the *plasmodium* parasite had a quinoline core. The first one was plasmoquine (**2**), which due to toxicity was replaced by primaquine (**3**) (Figure 1). In the same decade, chloroquine (**4**) started to be widely used, however, reports of resistance during the Vietnam War in the 1960's, led to the development of mefloquine (**5**), an effective anti-malarial drug for the next three decades. Again, subsequent reports of resistance and side effects narrowed its use. Even today, the quinoline core is still present in compounds in clinical trials such as ferroquine (**6**) and in approved drugs like amodiaquine (**7**) (Figure 1).

MATERIALS AND METHODS

THF was distilled from sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. Anhydrous DMF (98%) was used as purchased. Acetonitrile was HPLC grade. All other aquired solvents and reagents were used

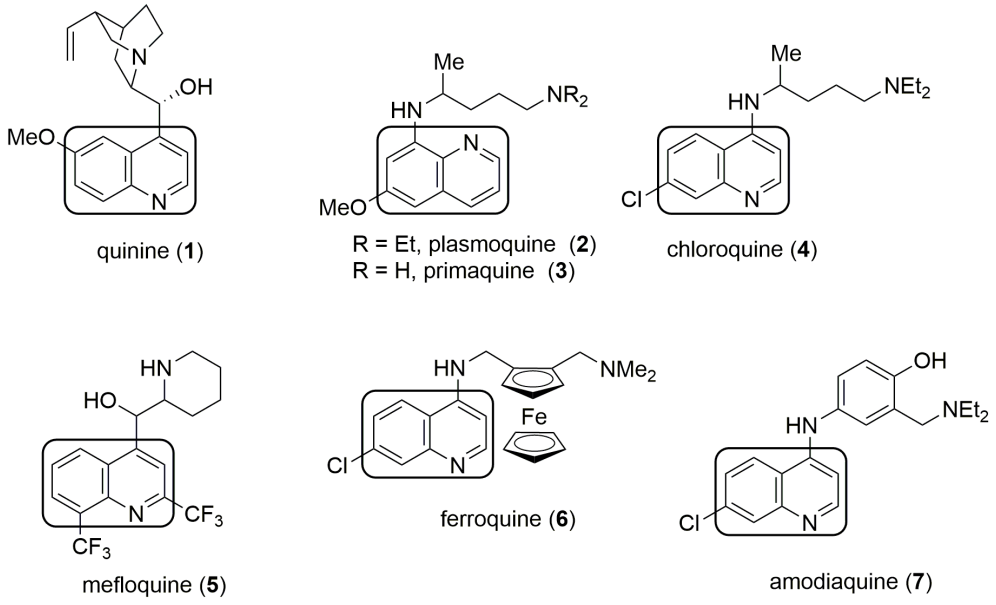


Figure 1 - Compounds containing quinoline heterocycle with antimalarial activity.

without further purification. Derivatives **15** and **16** were synthesized from the known ketones isopropyldihydro-2H-pyran-4(*3H*)-one (Nitz et al. 2009) and 2,6-dimethyltetrahydropyran-4-one (Reddy et al. 2004) respectively. For analytical purposes, derivative **14** was converted in solid hydrochloride salt using HCl 1 M in Et_2O solution. Compounds **18**, **21** and **28** were also converted in hydrochloride salts with the same procedure, to be used in the biological assay. Flash column chromatography was conducted using silica gel (230−400 mesh) or aluminum oxide (activated, neutral, Brockmann I, ~150 mesh). Analytical thin-layer chromatography was performed on silica gel 60 F254 plates and the visualization was accomplished using UV light or phosphomolybdic acid followed by heating. ^{1}H and proton-decoupled ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD or DMSO-d^6 in different magnetic fields as indicated. The chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) at 0.00 ppm or the solvent residual peak as an internal standard (7.26 ppm with $CDCl_3$, 3.31 ppm with CD_3OD and 2.50 ppm with DMSO-d^6 for ^{1}H NMR spectra and 77.0 ppm with $CDCl_3$, 49.0 ppm with CD_3OD and 39.52 ppm with DMSO-d^6 for ^{13}C NMR spectra). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet, a = aparent), coupling constant(s) in Hz, integration. High-resolution mass spectrometry (HRMS) was performed using the electrospray ionization (ESI) technique. The parent ions ($[M + H]^+$ or $[M + Na]^+$) are cited.

EXPERIMENTAL

General procedure A1, reductive amination

To a solution of the ketone/aldehyde (0.4 mmol, 1 eq) and amine (0.5 mmol, 1.3 eq) in dichloromethane (0.3 mL, 1.2 M) was added $NaBH(AcO)_3$ (0.45 mmol, 1.2 eq). The mixture was stirred overnight at room temperature under argon atmosphere. The reaction mixture was quenched by adding 0.5 M NaOH and stirred for 30 minutes. The product was extracted with dichloromethane and the organic layer was washed with brine and dried over $MgSO_4$. It was filtered and the solvent was evaporated. The crude was purified by column chromatography on silica gel using $CHCl_3$-MeOH (9:1, v/v) as eluent.

General procedure A2, reductive amination

A solution of aldehyde and amine was stirred in dichloromethane until the formation of the imine was confirmed by IR. Methanol and $NaBH_4$ were then added to the solution and it was stirred over night. The reaction was quenched with aq. sat. $NaHCO_3$ solution and stirred for 30min. It was extracted with dichloromethane and the combined organic layer were washed with brine and dried over $MgSO_4$. It was filtered and the solvent was evaporated. The crude was purified by column chromatography on silica gel using $CHCl_3$-MeOH (9:1, v/v) as eluent.

General procedure B, nucleophilic aromatic substitution

The aromatic halide (0.17 mmol) was dissolved in the cyclic amine (1 mL, 0.17 M). The mixture was heated to reflux until no more starting material was observed by TLC. The reaction mixture was concentrated *in vacuo*, taken in water and extracted with dichloromethane. The organic layers were washed with brine and dried over $MgSO_4$. After filtration, the solvent was evaporated and the crude was purified by column chromatography on silica gel using $CHCl_3$ as eluent.

General Procedure C, oxime synthesis (Niralwad et al. 2011)

A solution of aldehyde (1 mmol, 1.0 eq), hydroxylamine hydrochloride (1.5 mmol, 1.5 eq),

and sodium acetate (1.5 mmol, 1.5 eq) in ethanol (2.5 mL, 0.4 M) was stirred overnight at room temperature. After the completion of reaction as monitored by TLC, the solvent was evaporated and water was added. The mixture was extracted with dichloromethane, the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated to yield the pure product.

General procedure D, Oxime reduction

An access of Raney-Ni in water was washed with methanol and added to a solution of the oxime (0.2 mmol, 1 eq) and ammonia (7M in methanol, 1.2 mL, 43 eq) in methanol (4 mL, 0.05 M). The reaction vessel was flushed with hydrogen gas and stirred under hydrogen atmosphere until TLC confirmed consumption of starting material. The crude was filtered through a short silica plug, concentrated and purified by column chromatography on silica gel using $CHCl_3$-MeOH (9:1, v/v) as eluent.

2,2-dimethyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine (**12**)

Figure 3 - route 2

Intermediate 8: General procedure C, from aldehyde **9**, yield: quant.

Intermediate 10: General procedure B, yield: 77%.

Intermediate 11: General procedure D, yield: 83%

Derivative 12: General procedure A1, yield: 75%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.87 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 7.0 Hz, 1H), 7.12 (at, *J* = 7.5 Hz, 1H), 3.98 (q, *J* = 13.7 Hz, 2H), 3.82 - 3.76 (m, 1H), 3.73 (t, *J* = 6.4 Hz, 4H), 3.63 (td, *J* = 12.3, 2.1 Hz, 1H), 2.94 – 2.82 (m, 1H), 2.65 (s, 3H), 2.03 - 1.94 (m, 4H), 1.89 - 1.78 (m, 2H), 1.34 (ddd, *J* = 24.0, 12.3, 5.0 Hz, 1H), 1.25 (s, 5H), 1.19 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 156.2, 145.4, 137.5, 134.3, 129.0, 124.6, 123.6, 123.4, 121.9, 72.2, 60.7, 50.8, 49.6, 48.5, 44.2, 33.7, 31.6, 25.7, 22.6, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{22}H_{32}N_3O$ 354.2545, found 354.2532.

N-((8-methyl-2-(pyrrolidin-1-yl)quinolin-3-yl) methyl)tetrahydro-2H-pyran-4-amine **(14)**

Figure 3 - route 1

Intermediate 13: General procedure A2, from aldehyde **9**, yield: 84%

Derivative 14: General procedure B, product crystallized as salt with 1M HCl in Et_2O, yield: 68%. **^{1}H NMR (500 MHz, MeOD d_4, hydrochloride salt) δ =** 8.67 (s, 1 H), 7.79 (d, *J* = 7.9 Hz, 1 H), 7.72 (d, *J* = 7.3 Hz, 1 H), 7.47 (at, *J* = 7.7 Hz, 1 H), 4.72 (s, 2 H), 4.07 - 4.14 (m, 4 H), 4.03 (dd, *J* = 11.6, 4.4 Hz, 2 H), 3.65 (tt, *J* = 11.7, 4.2 Hz, 1 H), 3.41 - 3.51 (m, 2 H), 3.25 - 3.29 (m, 2 H), 2.68 (s, 3 H), 2.15 - 2.26 (m, 6 H), 1.83 (qd, *J* = 12.2, 4.7 Hz, 2 H).**^{13}C NMR (126 MHz, MeOD d_4, hydrochloride salt)** δ = 151.8, 149.5, 136.5, 136.2, 128.1, 127.2, 127.0, 122.0, 118.1, 67.0, 56.8, 53.0, 46.3, 30.6, 26.6, 15.9. **HRMS m/z:** $[M+H]^+$ calcd for $C_{20}H_{28}N_3O$ 326.2232, found 326.2220.

2-isopropyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(15)**

Figure 3 - route 2

Derivative 15: General procedure A1, from intermediate **11**, yield: 54%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.87 (s, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 6.9 Hz, 1H), 7.12 (at, *J* = 7.5 Hz, 1H), 3.93 (s, 2H), 3.83 (td, *J* = 11.7, 2.2 Hz, 1H), 3.79 - 3.71 (m, 5H), 3.47 - 3.36 (m, 1H), 3.13 - 3.04 (m, 1H), 2.65 (s, 3H), 2.03 - 1.94 (m, 4H), 1.85 - 1.74 (m, 2H), 1.66 - 1.60 (m, 1H), 1.57 - 1.48 (m, 2H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** = 156.1, 145.5, 138.1, 134.3, 129.0, 124.6, 123.6, 123.3, 121.9, 62.9, 50.3, 50.1, 49.5, 33.3, 32.5, 31.1, 25.7, 18.6, 18.4, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{23}H_{34}N_3O$ 368.2696, found 368.2688.

2,6-dimethyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(16)**

Figure 3 - route 2

Derivative 16: General procedure A1, from intermediate **11**, yield: 32%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.88 (s, 1H), 7.45 (t, *J* = 8.7 Hz, 1H), 7.38 (d, *J* = 6.9 Hz, 1H), 7.12 (at, *J* = 7.5 Hz, 1H), 3.98 – 3.84 (m, 2H), 3.93 (s, 2H), 3.74 (t, *J* = 6.6 Hz, 4H), 3.10 – 3.04 (m, 1H), 2.65 (s, 3H), 2.05 – 1.93 (m, 4H), 1.64 – 1.55 (m, 2H), 1.46 – 1.34 (m, 2H), 1.15 (d, *J* = 6.2 Hz, 6H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** = 156.2, 145.5, 137.9, 134.3, 129.0, 124.6, 123.7, 123.3, 121.9, 67.9, 51.1, 50.1, 49.5, 37.9, 25.7, 22.1, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{22}H_{32}N_3O$ 354.2545, found 354.2531.

1-methyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)piperidin-4-amine **(18)**

Figure 3 - route 1

Intermediate 17: General procedure A2, yield: quant.

Derivative 18: General procedure B, from intermediate **17** yield: 13%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.89 (s, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 6.9 Hz, 1H), 7.14 (at, *J* = 7.5 Hz, 1H), 3.99 (s, 2H), 3.75 (t, *J* = 6.5 Hz, 4H), 2.83 (d, *J* = 11.4 Hz, 2H), 2.67 (s, 3H), 2.55-2.47 (m, 1H), 2.28 (s, 3H), 2.05 - 1.96 (m, 6H), 1.92 (ad, *J* = 12.1 Hz, 2H), 1.54 -1.46 (m, 2H).**^{13}C NMR (126 MHz, $CDCl_3$) δ** = 156.2, 145.4, 137.6, 134.3, 128.9, 124.6, 123.7, 123.4, 121.9, 54.5, 53.9, 49.5, 48.9, 46.2, 32.8, 25.7, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{21}H_{31}N_4$ 339.2543, found 339.2535.

2,2,6,6-tetramethyl-N-((8-methyl-2-(pyrrolidin-1-yl)quinolin-3-yl)methyl)piperidin-4-amine **(20)**

Figure 3 - route 1

Intermediate 19: General procedure A2, yield: quant.

Derivative 20: General procedure B, from intermediate **19**, yield: quant. **^{1}H NMR (500 MHz, $CDCl_3$) δ** = 7.88 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 6.9 Hz, 1H), 7.12 (at, *J* = 7.5 Hz, 1H), 3.98 (s, 2H), 3.71 (t, *J* = 6.4 Hz, 4H), 3.06 – 2.97 (m, 1H), 2.64 (s, 3H), 1.98 (t, *J* = 6.5 Hz, 4H), 1.93 (dd, *J* = 13.5, 2.8 Hz, 2H), 1.69 (s, 6H), 1.63 (t, *J* = 12.5 Hz, 2H), 1.48 (s, 6H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 156.1, 145.5, 137.8, 134.3, 129.1, 124.7, 123.4, 123.1, 122.0, 57.5, 49.7, 48.7, 47.8, 42.9, 30.9, 25.8, 25.7, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{24}H_{37}N_4$ 381.3013, found 381.3004.

3,3-dimethyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)cyclohexanamine **(21)**

Figure 3 - route 2

Derivative 21: General procedure A1, from intermediate **11**, yield: 80%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.87 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 6.9 Hz, 1H), 7.15 – 7.07 (m, 1H), 4.03 – 3.90 (m, 2H), 3.75 - 3.71 (m, 4H), 2.70 – 2.60 (m, 1H), 2.64 (s, 3H), 2.03 – 1.94 (m, 4H), 1.69 – 1.57 (m, 2H), 1.49 – 1.37 (m, 2H), 1.32 – 1.36 (m, 1H), 1.11 (td, *J* = 13.4, 4.1 Hz, 1H), 1.03 – 0.95 (m, 2H), 0.94 (d, *J* = 5.0 Hz, 3H), 0.89 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 156.2, 145.4, 137.5, 134.2, 128.8, 124.6, 124.0, 123.4, 121.8, 53.2, 49.5, 49.1, 46.9, 39.0, 33.8, 33.3, 31.4, 25.7, 25.2, 21.4, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{23}H_{34}N_3$ 352.2747, found 352.2738.

2-methyl-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydrofuran-3-amine **(22)**

Figure 3 - route 2

Derivative 22: General procedure A1, from intermediate **11**, yield: 56%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.88 (s, 1 H), 7.46 (d, *J* = 7.9 Hz, 1 H), 7.38 (d, *J* = 6.9 Hz, 1 H), 7.12 (at, *J* = 7.5 Hz, 1 H), 4.04 (d, *J* = 14.0 Hz, 1 H), 3.87 - 4.01 (m, 2 H), 3.85 (d, *J* = 14.0 Hz, 1 H), 3.66 - 3.78 (m, 5 H), 3.17 (q, *J* = 5.1 Hz, 1 H), 2.65 (s, 3 H), 2.04-2.10 (m, 1 H), 1.93 - 2.02 (m, 4 H), 1.84-1.91 (m, 1 H), 1.25 (d, *J* = 6.6 Hz, 1 H), 1.22 (d, *J* = 6.4 Hz, 3 H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 156.2, 145.4, 137.6, 134.3, 129.0, 124.6, 123.4, 123.3, 122.0, 77.1, 65.7, 59.5, 50.1, 49.5, 32.1, 25.6, 17.7, 14.8. **HRMS m/z:** $[M+H]^+$ calcd for $C_{20}H_{28}N_3O$ 326.2226, found 326.2221.

8-methyl-3-((4-methylpiperazin-1-yl)methyl)-2-(pyrrolidin-1-yl)quinoline **(24)**

Figure 3 - route 2

Intermediate 23: General procedure A1, from aldehyde **9**, yield: 83%

Derivative 24: General procedure B, yield: 88%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.81 (s, 1 H), 7.44 (d, *J* = 7.9 Hz, 1 H), 7.37 (d, *J* = 6.9 Hz, 1 H), 7.09 (at, *J* = 7.5 Hz, 1 H), 3.76 - 3.84 (m, 4 H), 3.63 (s, 2 H), 2.63 (s, 3 H), 2.47 (br, 8 H), 2.29 (s, 3 H), 1.91 - 2.00 (m, 4 H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = ppm 156.3, 145.7, 139.3, 134.0, 129.0, 124.7, 122.8, 121.5, 120.5, 60.6, 55.3, 52.9, 49.4, 46.0, 25.7, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{20}H_{29}N_4$ 325.2387, found 325.2379.

N1, N1-dimethyl-N2-((8-methyl-2-(pyrrolidin-1-yl)quinolin-3-yl)methyl)ethane-1,2-diamine **(26)**

Figure 3 - route 2

Intermediate 25: General procedure A2, from aldehyde **9**, crude product submitted to next step without purification.

Derivative 26: General procedure B, yield: 45% over two steps. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.88 (s, 1 H), 7.46 (d, *J* = 7.8 Hz, 1 H), 7.38 (d, *J* = 7.0 Hz, 1 H), 7.12 (at, *J* = 7.5 Hz, 1 H), 4.00 (s, 2 H), 3.66 - 3.72 (m, 4 H), 2.70 (t, *J* = 6.0 Hz, 2 H), 2.64 (s, 3 H), 2.46 (t, *J* = 6.0 Hz, 2 H), 2.21 (s, 6 H), 1.93 - 2.01 (m, 4 H).**^{13}C NMR (126 MHz, $CDCl_3$), δ** =156.2, 145.4, 137.7, 134.3, 128.9, 124.7, 123.4, 122.9, 122.0, 58.7, 51.7, 49.5, 46.3, 45.3, 25.6, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{19}H_{29}N_4$ 313.2387, found 313.2378.

3-methoxy-N-((8-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)propan-1-amine **(28)**

Figure 3 - route 2

Intermediate 27: General procedure A2, from aldehyde **9**, yield: quant.

Derivative 28: General procedure B, yield: 13%. **^{1}H NMR (400 MHz, $CDCl_3$) δ =** 7.85 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 7.0 Hz, 1H), 7.18 – 7.05 (m, 1H), 3.95 (s, 2H), 3.79 – 3.65 (m, 4H), 3.45 (t, *J* = 6.2 Hz, 2H), 3.32 (s, 3H), 2.72 (t, *J* = 6.8 Hz, 2H), 2.65 (s, 3H), 2.07 – 1.93 (m, 4H), 1.79 (p, *J* = 6.5 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ =** 156.2, 145.4, 137.6, 134.3, 128.9, 124.7, 123.34, 123.32, 121.8, 71.3, 58.6, 52.0, 49.5, 46.8, 30.0, 25.7, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{19}H_{28}N_3O$ 314.2232, found 314.2217.

2,2-dimethyl-N-((8-methyl-2-(piperidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(31)**

Figure 3 - route 2

Intermediate 29: General procedure B from oxime **8**, yield: 91%

Intermediate 30: General procedure D, yield: 85%

Derivative 31: General procedure A1, yield: 21%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.98 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 7.0 Hz, 1H), 7.26 - 7.22 (m, 1H), 3.94 (q, *J* = 13.5 Hz, 2H), 3.79 (ddd, *J* = 12.0, 4.9, 1.8 Hz, 1H), 3.63 (td, *J* = 12.3, 2.2 Hz, 1H), 3.32 - 3.23 (m, 4H), 2.84 (tt, *J* = 11.3, 4.1 Hz, 1H), 2.71 (s, 3H), 1.84 (dddd, *J* = 18.7, 14.6, 4.0, 2.0 Hz, 2H), 1.79 - 1.73 (m, 5H), 1.67 (dd, *J* = 11.2, 6.1 Hz, 2H), 1.34 (ddd, *J* = 23.9, 12.5, 5.0 Hz, 1H), 1.26 (s, 4H), 1.18 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 160.3, 145.2, 137.3, 135.7, 128.9, 127.5, 125.3, 124.8, 123.9, 72.3, 60.7, 51.6, 50.7, 47.3, 44.2, 33.6, 31.7, 26.4, 24.8, 22.6, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{23}H_{34}N_3O$ 368.2696, found 368.2690.

2,2-dimethyl-N-((8-methyl-2-morpholinoquinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(34)**

Figure 3 - route 2

Intermediate 32: General procedure B, from oxime **8**, yield: 77%

Intermediate 33: General procedure D, yield: 91%

Derivative 34: General procedure A1, yield: 56%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 8.03 (s, 1 H), 7.54 (d, *J* = 7.9 Hz, 1 H), 7.45 (d, *J* = 7.0 Hz, 1 H), 7.27 (at, *J* = 7.5 Hz, 1 H), 3.87 - 3.99 (m, 6 H), 3.80 (ddd, *J* = 12.1, 4.9, 1.7 Hz, 1 H), 3.59 - 3.69 (m, 1 H), 3.35 - 3.45 (m, 4 H), 2.88 (tt, *J* = 11.3, 4.1 Hz, 1 H), 2.71 (s, 3 H), 1.79 - 1.92 (m, 2 H), 1.65 (br, 2 H, NH + H_2O), 1.29 - 1.39 (m, 1 H), 1.22 - 1.29 (m, 4 H), 1.16 - 1.22 (m, 3 H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** =159.0, 145.1, 137.9, 135.8, 129.1, 126.9, 125.4, 124.8, 124.3, 72.2,

67.1, 60.6, 50.9, 50.8, 47.0, 44.1, 33.6, 31.6, 22.6, 17.7. **HRMS m/z:** $[M+H]^+$ calcd for $C_{22}H_{32}N_3O_2$ 370.2489, found 370.2482.

2,2-dimethyl-N-((8-methyl-2-(4-methylpiperazin-1-yl)quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(37)**

Figure 3 - route 2

Intermediate 35: General procedure B, from oxime **8**, yield: 89%

Intermediate 36: General procedure D, yield: 68%

Derivative 37: General procedure A1, yield: 57%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 8.03 (s, 1 H), 7.56 (d, *J* = 7.9 Hz, 1 H), 7.47 (d, *J* = 7.0 Hz, 1 H), 7.25 - 7.32 (m, 1 H), 3.97 (q, *J* = 13.4 Hz, 2 H), 3.79 - 3.87 (m, 1 H), 3.67 (td, *J* = 12.2, 2.1 Hz, 1 H), 3.37 - 3.53 (m, 4 H), 2.89 (att, *J* = 11.3, 4.1 Hz, 1 H), 2.73 (s, 3 H), 2.67 (br, 4 H), 2.42 (s, 3 H), 1.83 - 1.93 (m, 2 H), 1.72 (br, 2 H, NH + H_2O), 1.37 (qd, *J* = 12.1, 5.0 Hz, 1 H), 1.25 - 1.32 (m, 4 H), 1.22 (s, 3 H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 159.2, 145.1, 137.6, 135.8, 129.0, 127.0, 125.3, 124.7, 124.1, 72.2, 60.7, 55.4, 50.8, 50.1, 47.1, 46.3, 44.1, 33.6, 31.6, 22.6, 17.6. **HRMS m/z:** $[M+H]^+$ calcd for $C_{23}H_{35}N_4O$ 383.2805, found 383.2796.

2,2-dimethyl-N-((8-methylquinolin-3-yl)methyl) tetrahydro-2H-pyran-4-amine **(39)**

Intermediate 38: To the oxime **8** (77 mg) in methanol (4mL) was added 10% Pd/C (8 mg) and the reaction was purged with hydrogen gas and stirred under hydrogen atmosphere over night. After filtration through a short silica plug, the crude material was treated with 1M HCl in ether to obtain the product as salt. Yield: 98%

Derivative 39: General procedure A1, yield: 40%. **^{1}H NMR (250 MHz, $CDCl_3$) δ** = 8.91 (d, *J* = 2.1 Hz, 1 H), 8.08 (d, *J* = 2.2 Hz, 1 H), 7.65 (d, *J* = 7.9 Hz, 1 H), 7.54 (d, *J* = 6.6 Hz, 1 H), 7.43 (at, *J* = 7.7 Hz, 1 H), 4.04 (s, 2 H), 3.74 - 3.88 (m, 1 H), 3.64 (td, *J* = 12.2, 2.2 Hz, 1 H), 2.93 (tt, *J* = 11.3, 4.1 Hz, 1 H), 2.82 (s, 3 H), 1.78 - 1.97 (m, 2 H), 1.51 (br, 3 H, NH + H_2O), 1.28 - 1.45 (m, 2 H), 1.26 (s, 3 H), 1.19 (s, 3 H). **HRMS m/z:** $[M+H]^+$ calcd for $C_{18}H_{25}N_2O$ 285.1967, found 285.1956.

3-(((2,2-dimethyltetrahydro-2H-pyran-4-yl)amino) methyl)-N,8-dimethylquinolin-2-amine **(43)**

Intermediate 40: A solution of aldehyde **9** (50 mg, 0.24 mmol) and methylamine (40% in water, 0.21 mL, 0.24 mmol) in dioxane (1.5 mL) was heated in a microwave oven for 10min at 120°C followed by 45min at 100°C. The solvent was evaporated and the crude stirred in THF/1 M HCl (1:1) for 1h. After evaporation of volatiles the residue was neutralized with aq. sat. $NaHCO_3$ and extracted with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by chromatography over silica gel with Hexanes-Ethyl acetate (99:1, v/v) to yield: 87%.

Intermediate 41: General procedure C, yield: quant.

Intermediate 42: General procedure D, yield: 36%

Derivative 43: General procedure A1, yield: 25%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 7.55 (s, 1H), 7.41 – 7.36 (m, 2H), 7.13 (s, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 3.91 (s, 2H), 3.79 (ddd, *J* = 12.0, 5.0, 1.6 Hz, 1H), 3.62 (td, *J* = 12.4, 2.2 Hz, 1H), 3.12 (s, 3H), 2.83 (tt, *J* = 11.3, 4.0 Hz, 1H), 2.68 (s, 3H), 1.92-1.88 (m, 1H), 1.86-1.83 (m, 1H), 1.33 – 1.24 (m, 5H), 1.20 – 1.15 (m, 3H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 156.86, 146.40, 135.51, 133.91, 129.14, 124.86, 122.73, 121.29, 121.02, 60.69, 50.18, 49.31, 44.11, 33.59, 31.67, 27.93, 22.52, 17.81, 0.04. **HRMS m/z**: $[M+H]^+$ calcd for $C_{19}H_{28}N_3O$ 314.2232, found 314.2228.

3-(((2,2-dimethyltetrahydro-2H-pyran-4-yl)amino) methyl)-N,N,8-trimethylquinolin-2-amine (**49)**

Intermediate 44: To a solution of the 8-methyl-2-(methylamino)quinoline-3-carbaldehyde **(40)** (184 mg, 0.96 mmol) and ethane-1,2-diol (0.23 mL, 4.10 mmol) in toluene (1.5 mL), TsOH (16 mg, 0.09 mmol) was added at room temperature. The mixture was refluxed until no more starting material was observed by TLC. The reaction was

allowed to reach room temperature and quenched by addition of 50 mL of water. After stirring for 10 minutes, the crude was extracted with ethyl acetate. The combined organic layers were washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude was purified by column chromatography on silica gel using Hexanes-Ethyl acetate (95:5, v/v) as eluent. Yield: 91%

Intermediate 45: A DMF suspension (2.5 mL) of compound **44** (214 mg, 0.88 mmol), 60% sodium hydride (53 mg, 1.31 mmol) and methyl iodide (0.066 mL, 1.00 mmol) was stirred for 2 hours at room temperature. Water was added to the reaction mixture and it was extracted with ethyl acetate. The organic layer was washed (sequentially with water and brine) and dried ($MgSO_4$). The solvent was evaporated and the crude was purified by column chromatography on silica gel using Hexanes-Ethyl acetate (95:5, v/v) as eluent. Yield: 88%

Intermediate 46: A suspension of compound **45** (167 mg, 0.17 mmol) in HCl 1M (3mL) was stirred at room temperature for 1 hour. The crude was neutralized with $NaHCO_3$ aqueous and extracted with ethyl acetate, washed with brine and dried ($MgSO_4$). The crude was used without further purification Yield: 95%

Intermediate 47: General procedure C, yield: 88%

Intermediate 48: General procedure D, yield: 81%

Derivative 49: General procedure A1, yield: 63%. **^{1}H NMR (500 MHz, $CDCl_3$) δ =** 8.01 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 7.0 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 4.02 – 3.92 (m, 2H), 3.83 – 3.76 (m, 1H), 3.63 (td, *J* = 12.3, 2.0 Hz, 1H), 3.01 (s, 7H), 2.87 (tt, *J* = 11.4, 4.1 Hz, 1H), 2.71 (s, 3H), 1.85 (ddd, *J* = 12.7, 3.8, 1.9 Hz, 2H), 1.51 (s, 1H), 1.34 (ddd, *J* = 24.0, 12.4, 5.1 Hz, 1H), 1.29 – 1.22 (m, 5H), 1.19 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$), δ** = 159.79, 144.93, 137.37, 135.30, 128.95, 126.42, 124.88, 124.73, 123.45, 72.28, 60.74, 50.85, 47.43, 44.22, 42.08, 33.71, 31.68, 22.62, 17.66. **HRMS m/z**: $[M+H]^+$ calcd for $C_{20}H_{30}N_3O$ 328.2389, found 328.2380.

2,2-dimethyl-N-((2-(pyrrolidin-1-yl)quinolin-3-yl) methyl)tetrahydro-2H-pyran-4-amine **(53)**

Intermediate 50: Commercially available 2-chloro-3-quinolinecarboxaldehyde was submitted to general procedure C, yield: 85%

Intermediate 51: General procedure B, yield: 85%

Intermediate 52: General procedure D, yield: 40%

Derivative 53: General procedure A1, yield: 92%. **^{1}H NMR (250 MHz, $CDCl_3$) δ** = 7.91 (s, 1 H), 7.72 (d, *J* = 8.4 Hz, 1 H), 7.59 (d, *J* = 7.9 Hz, 1 H), 7.44 - 7.55 (m, 1 H), 7.15 - 7.25 (m, 1 H), 3.87 - 4.07 (m, 2 H), 3.52 - 3.87 (m, 6 H), 2.87 (tt, *J* = 11.3, 4.0 Hz, 1 H), 1.90 - 2.07 (m, 4 H), 1.74 - 1.90 (m, 2 H), 1.53 (br, 2 H, NH + H_2O), 1.22 - 1.42 (m, 5 H), 1.18 (s, 3 H). **^{13}C NMR (63 MHz, $CDCl_3$), δ** = 157.4, 146.7, 137.2, 128.9, 126.8, 126.3, 124.4, 123.8, 122.3, 72.2, 60.7, 50.8, 49.7, 48.5, 44.2, 33.7, 31.6, 25.6, 22.5. **HRMS m/z:** $[M+H]^+$ calcd for $C_{21}H_{30}N_3O$ 340.2383, found 340.2374.

2,2-dimethyl-N-((7-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(57)**

Intermediate 54: Commercially available 2-chloro-7-methyl-3-quinolinecarboxaldehyde was submitted to general procedure C, crude product used in next step without purification.

Intermediate 55: General procedure B, yield: 82%

Intermediate 56: General procedure D, yield: 98%

Derivative 57: General procedure A1, yield: 63%. **^{1}H NMR (500 MHz, $CDCl_3$) δ** = 7.86 (s, 1H), 7.53 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.5 Hz, 1H), 3.95 (q, *J* = 13.8 Hz, 2H), 3.78 (ddd, *J* = 12.0, 5.0, 1.8 Hz, 1H), 3.69 (s, 4H), 3.65 – 3.58 (m, 1H), 2.86 (tt, *J* = 11.4, 4.1 Hz, 1H), 2.47 (s, 3H), 2.01 – 1.93 (m, 4H), 1.88 – 1.77 (m, 2H), 1.39 – 1.21 (m, 2H), 1.24 (s, 3H), 1.18 (s, 3H). **^{13}C NMR (126MHz, $CDCl_3$) δ** = 157.5, 146.8, 139.1, 137.1, 126.4, 125.7, 124.5, 123.4, 121.8, 72.2, 60.7, 50.8, 49.7, 48.6, 44.1, 33.7, 31.6, 25.6, 22.6, 21.8. **HRMS m/z**: [M+H]+ calcd for $C_{22}H_{32}N_3O$ 354.2545, found 354.2533.

2,2-dimethyl-N-((6-methyl-2-(pyrrolidin-1-yl) quinolin-3-yl)methyl)tetrahydro-2H-pyran-4-amine **(61)**

Intermediate 58: Commercially available 2-chloro-6-methyl-3-quinolinecarboxaldehyde was submitted to general procedure C, yield: 57%
Intermediate 59: General procedure B, yield: 99%
Intermediate 60: General procedure D, yield: 93%
Derivative 61: General procedure A1, yield: 30%. **^{1}H NMR (250 MHz, $CDCl_3$) δ** = 7.83 (s, 1 H), 7.63 (d, *J* = 8.4 Hz, 1 H), 7.29 - 7.40 (m, 2 H), 3.87 - 4.02 (m, 2 H), 3.55 - 3.84 (m, 6 H), 2.85 (tt, *J* = 11.3, 4.1 Hz, 1 H), 2.44 (s, 3 H), 1.90 - 2.01 (m, 4 H), 1.73 - 1.90 (m, 2 H), 1.54 (br, 2 H, NH + H_2O), 1.20 - 1.43 (m, 5 H), 1.18 (s, 3 H). **HRMS m/z:** $[M+H]^+$ calcd for $C_{22}H_{32}N_3O$ 354.2540, found 354.2532.

N-((8-chloro-2-(pyrrolidin-1-yl)quinolin-3-yl) methyl)-2,2-dimethyltetrahydro-2H-pyran-4-amine **(65)**

Intermediate 62: To the stirred solution of 2, 8-dichloroquinoline (2.0 g, 10.10 mmol) in THF under Argon (20 mL), freshly prepared LDA (3.78 g, 35.35 mmol) was added at -78 °C and the mixture was stirred for 2 h at the same temperature. Then, DMF (2.9 g, 40.40 mmol) was added to the reaction mixture and stirred for 2 h at -78 °C. The resulting reaction mixture was quenched with NH_4Cl solution and extracted with ethyl acetate (3x20 mL). The organics layers were washed with brine and concentrated. The concentrated product was purified through silica gel column chromatography using 10% ethyl acetate in pet ether to afford step1 product (0.9 g, 39%) as pale yellow liquid.
Intermediate 64: General procedure A1, yield: 47%.
Derivative 65: NaO^tBu (0.3 g, 2.96 mmol) was added to stirred solution of step 2 product (0.4 g, 1.18 mmol) and pyrrolidine (0.17 g, 2.36 mmol) in 1, 4-dioxane (10 mL) at room temperature and stirred for 5 h at 80 °C. Then, the reaction mixture was concentrated under reduced pressure to obtain a residue. The resulting residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The concentrated product was purified through silica gel column chromatography using 5% methanol in dichloromentahne followed by preparative HPLC.Yield: 38% as off white solid. **^{1}H NMR (400 MHz, DMSO-d_6)** = δ 8.77 (brs, 1H), 8.22 (s, 1H), 7.77 (d, *J* = 8 Hz, 1H), 7.71 (d, *J* = 8 Hz, 1H), 7.27-7.23 (m, 1H), 4.47 (brs, 2H), 3.55-3.77 (m, 7H), 1.96-2.04 (m, 6H), 1.53-1.38 (m, 2H), 1.21 (s, 3H), 1.19 (s, 3H). **^{13}C NMR (100 MHz, DMSO-d_6) δ** = 156.2, 143.04, 139.92, 130.38, 129.47, 126.96, 124.09, 122.83, 117.96, 72.04, 59.38, 52.80, 50.28, 45.21, 31.74, 29.13, 25.74, 22.16. **HRMS m/z**: $[M+H]^+$ calcd for $C_{21}H_{29}ClN_3O$ 374.1999, found for 374.2100.

3-(((2,2-dimethyltetrahydro-2H-pyran-4-yl)amino) methyl)-2-(pyrrolidin-1-yl)quinolin-8-ol **(66)**

To a stirred solution of compound **65** (50 mg, 0.13mmol) in 1,4-dioxane (8 mL) was added NaO^tBu (18 mg, 0.18mmol) and 10% sodium hydroxide solution (0.5 mL) at room temperature. Then, it was degassed for 5 minutes. ^{t}Butyl Brett-Phos (2.28 mg, 0.02mmol) was added at RT and again degassed for 5 minutes. Then, it was heated to 100 °C for 16 h under sealed tube. After completion of the reaction, the reaction mixture was diluted with water (30 mL), extracted with ethyl acetate (2x50 mL). The resulting extracted ethyl acetate was dried over Na_2SO_4 and concentrated under vacuum. The resulting concentrated product was purified through prep-HPLC (0.1% HCOOH in acetonitrile). Yield: 21% as off white solid. **^{1}H NMR (400 MHz, DMSO- d_6)** δ = 8.01 (s, 1H),7.18 (d, 1H, *J* = 8 Hz), 7.14-7.10 (m, 1H), 6.94 (d, 1H, *J* = 7.6 Hz), 4.05 (d, 2H, *J* = 4.8 Hz); 3.75-3.69 (m, 6H), 2.78-2.92 (m, 1H), 2.06-2.02 (m, 4H), 1.94-1.88 (m, 2H), 1.38-1.27 (m, 2H), 1.24 (s, 3H), 1.20 (s, 3H).

Parasite reduction ratio assay

The intraerythrocytic *P. falciparum* growth inhibition by drugs was determined using a modification of the *in vitro* [3H]-hypoxanthine incorporation method and has been published previously (Sanz et al. 2012).

Malaria activity assay

The biological activities (IC_{50}) of compounds were determined against the drug sensitive *P. falciparum* NF54 strain *in vitro* (Desjardins et al. 1979). Additional resistance studies were conducted using the same protocol against various field-derived resistant strains which are described in Tables IV-V.

Metabolic stability assay

In vitro intrinsic clearance (CL_{int}) was assessed by substrate depletion upon incubation with human liver microsomes (HLM) and rat liver hepatocytes (Rat Heps). These assays, unless otherwise stated, were conducted in triplicate according to previously published procedure (Jones et al. 2017). Data are summarized in Tables I-III.

RESULTS AND DISCUSSION

The current work had the objective to find a tractable lead which could be transformed into a long acting drug suitable for Single Exposure Radical Cure and Prophylaxis (Burrows et al. 2013) when administered in combination with other anti-malarials. Similarly to a previous described series (Krake et al. 2017), the initial hit **12** was discovered through HTS (high throughput screening) and constitutes a trisubstituted quinoline with excellent anti-plasmodial activity (IC_{50} *Pf* NF54 = 22 nM). (Figure 2) Although this quinoline-based compound showed other interesting properties such as low cytotoxicity (IC_{50} THP1 > 50 μM; IC_{50} Hep G2 > 10 μM), minimal hERG inhibition (IC_{50} hERG = 23 μM) and good permeability in Caco2 cells (34 . 10^{-6} cm/s at pH = 6.5), a high logD led to metabolically instability and low solubility. MetID studies in human and mouse liver microsomes revealed that the main metabolite resulted from oxidation of 8-methyl substituent.

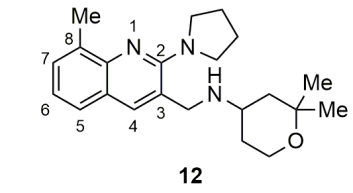
Regarding activity during the malaria life cycle, compound **12** acts only at the blood stage of the protozoan. The *in vitro* speed of killing was compared to known antimalarial drugs by means of a parasite reduction ration assay (Sanz et al. 2012). A moderate rate of killing was observed for **12** (Figure 2), which is uncommon for quinoline antimalarials and may indicate a different mode of action compared to marketed quinolines. It was also observed that this compound had significant loss in potency against the resistant strain *Pf* K1, which was a concern (Figure 2).

In view of this profile, our initial strategy was to reduce logD by removing hydrophobic moieties and introducing polar groups on the assumption this would lead to improved metabolic stability and solubility. We also hoped to increase activity against PfK1, and other resistant strains if necessary.

CHEMISTRY

For the synthesis of most analogs in this series (Figure 3), commercially available **9** was subjected to reductive amination with various amines, followed by aromatic nucleophilic substitution to form trissubsstituted quinolines (Route 1). In cases where R^1 was introduced from a ketone, **9** was first converted into the oxime **8** and reduced to the corresponding amine using Raney-Ni, followed by reductive amination to form the desired final product (Route 2). Compound **39**, a 3,8-dissubstituted derivative, without any group at the 2-position was prepared using Pd catalysed hydrogenolysis which reduced the oxime and cleaved the C-Cl bond in a one pot procedure.

8-chloro and 8-hydroxy derivatives **65** and **66** were accessed from commercially available



12

IC_{50} (*Pf* NF54 / *Pf* K1): 22 nM / 173 nM
$LogD_{pH\ 7.4}$: 3.8
$Solubility_{pH\ 7.4}$: 29 µM
Perm. Caco2: 34 . 10^{-6} cm/s
IC_{50} THP1: >50 µM
IC_{50} hERG: 23 µM
H Mics CLint: 102 µL/min/mg
Rat Heps CLint: >300 µL/min/10^{6}

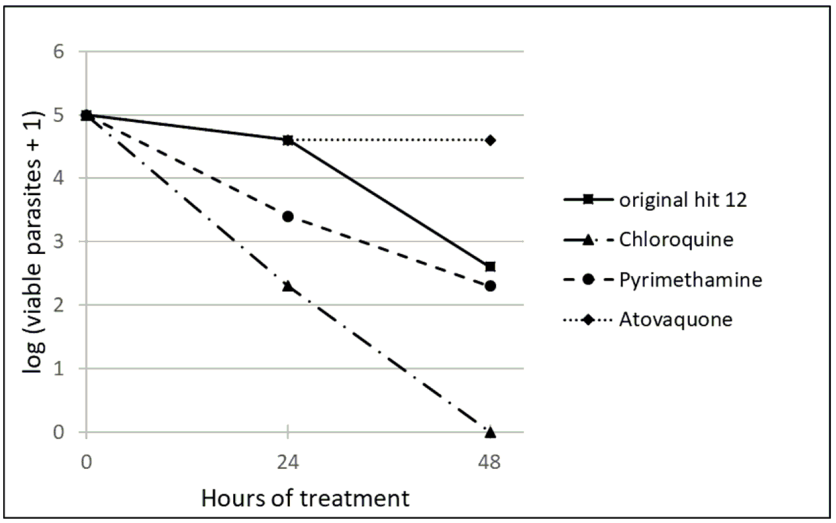


Figure 2 - Original Hit profile (left) and speed of killing plot (right) of viable parasites after treatment with antimalarial drugs and compound **12**.

starting material **63**, which was formylated and then underwent a similar reductive amination and aromatic substitution sequence as described before. The hydroxyl group of **66** was introduced last via palladium catalyzed hydroxylation (Figure 4).

SAR ANALYSIS

In order to explore SARs against *Plasmodium falciparum* (NF54 strain), we first investigated modifications of the tetrahydropyran unit (Table I). By removing both *gem*-dimethyls at the carbon α-Oxygen, we prepared **14**, a less lipophilic analogue with better metabolic stability in human microsomes, but inactive. Replacement of the *gem*-dimethyl with a bulkier isopropyl group afforded **15**. Although we did not expect to improve logD, this modification was performed to explore the influence of steric hindrance at this position, but all properties were inferior to the original hit **12**. Moving one of the tetrahydropyran methy groups around the ring in **16**, resulted in a moderately active compound but proved to be deleterious for solubility. Replacement of the oxygen by nitrogen afforded compounds **18** and **20** that were more soluble as a consequence of the introduction of a basic centre, with substantially enhanced metabolic stability for the *N*-Me piperidine analogue **18**, although its activity was reduced to the micromolar range. Evaluating the influence of a cyclohexane ring in comparison with tetrahydropyran, we synthesized quinoline **21**, which displayed moderate potency (200 nM). Thus, reduced activity for nitrogen or carbon analogues of **12** confirmed the importance of the oxygen in this part of the molecule for activity against *plasmodium*, possibly via a specific hydrogen bond with the target protein. However, reduction of ring size to the tetrahydrofuran analog **22** resulted in >50-fold drop of activity. Reducing the length of the linker through the piperazine **24** led to a complete loss of potency, athough logD, clearance and solubility were much improved. Using an acyclic substituent with nitrogen or oxygen at the end of the chain (derivatives **26** and **28**) significantly improved solubility for both analogues and the metabolic stability in human microsomes for the former, but none retained activity. Generally, we observed that modifications to the tetrahydropyran ring only impacted on solubility or clearance, with good metabolic stability for nitrogen-containing

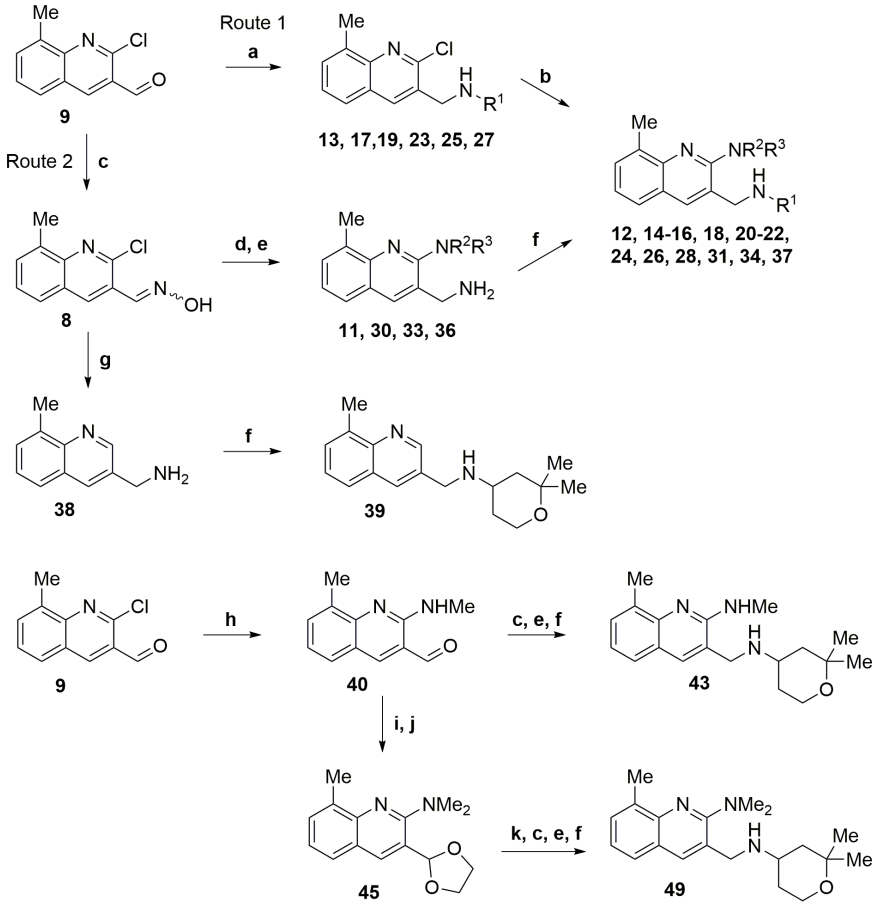


Figure 3 - General strategies for the synthesis of quinoline derivatives. Reagents and conditions: **a**) primary amine, CH_2Cl_2, then $NaBH_4$, MeOH, 83%-quant.; **b**) pyrrolidine, 100 °C, 13%-quant.; **c**) $H_2NOH.HCl$, NaOAc, EtOH, quant.; **d**) amine, 100 °C, 77%-91%; **e**) Raney-Ni, NH_3, MeOH, H_2, 40%-91%; **f**) ketone, $NaBH(OAc)_3$, CH_2Cl_2, 16%-92%; **g**) Pd/C, H_2, MeOH, then HCl/Et_2O, 98%; **h**) aq. NH_2CH_3, 1,4-dioxane, MW (microwave irradiation), 10 min/120 °C, 45 min/160 °C, 75%; **i**) ethylene glycol, p-TsOH, toluene, reflux, 91%; **j**) CH_3I, NaH, DMF (dimethylformamide), 0 °C, 88%; **k**) HCl 1M, rt, 95%.

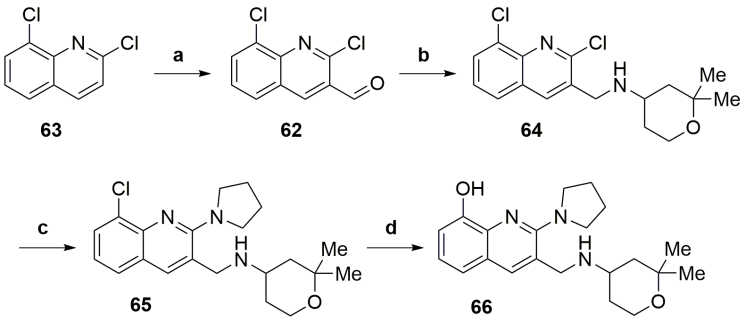
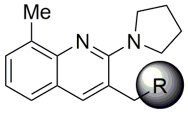
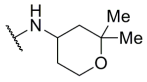
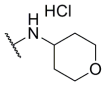
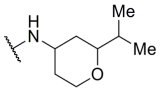
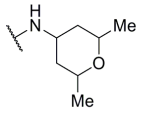
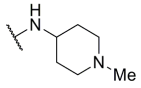
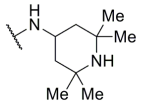
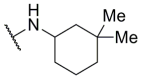
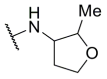
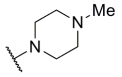
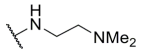
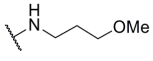


Figure 4 - Synthesis of 8-chloro and 8-hydroxy analogues. Reagents and conditions: **a**) LDA (Lithium diisopropylamide), THF, DMF, -78°C, 39%; **b**) 2,2-dimethyltetrahydro-2H-pyran-4-amine, $NaBH(OAc)_3$, DCE (1,2-dichloroethane), 47%; **c**) pyrrolidine, NaOtBu, dioxane, 80°C, 38%; **d**) $Pd_2(dba)_3$, NaOtBu, $NaOH_{aq}$ 10%, ^{t}Bu Brett Phos [2-(Di-*tert*-butylphosphino)-2′,4′,6′-triisopropyl-3,6-dimethoxy-1,1′-biphenyl], 1,4-dioxane, 100°C, 21%.

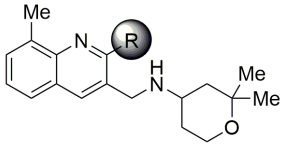
TABLE I
Selected modifications at the tetrahydropyran fragment.

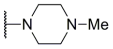


R	IC_{50} NF54 (nM)	$logD_{7.4}$	Solub. (µM)	H Mics CL_{int} (µl/min/mg)	Rat Heps CL_{int} (µl/min/10^6)
 **12**	22	3.8	29	102	>300
 **14**	5974	3.5	211	65	>300
 **15**	654	5.3	2	195	237
 **16**	74	4.0	4	205	ND
 **18**	4343	2.4	312	<3	59
 **20**	5962	2.1	324	107	84
 **21**	228	4.1	1	42	>300
 **22**	947	ND	ND	ND	ND
 **24**	>10000	3.6	349	29	ND
 **26**	6357	2.6	334	27	>300
 **28**	>10000	3.3	520	133	>300

Solub = solubility in phosphate buffer solution (PBS); H Mics CL_{int} = intrinsic human microsomal clearance; Rat Heps CL_{int} = intrinsic rat hepatic clearance; ND = not determined.

TABLE II
Influence of the 2-substituent at the quinoline heterocycle.



R	IC_{50} NF54 (nM)	$logD_{7.4}$	Solub. (μM)	H Mics CL_{int} (μl/min/mg)	Rat Heps CL_{int} (μl/min/10^6)
 **12**	22	3.8	29	102	>300
 **31**	168	4.1	47	65	292
 **34**	5885	3.1	504	77	155
 **37**	9737	2.5	589	64	>300
 **39**	>10000	2.1	671	7	153
 **43**	>10000	3.2	341	52	175
 **49**	3156	3.2	824	48	>300

Solub = solubility in PBS; H Mics CL_{int} = intrinsic human microsomal clearance; Rat Heps CL_{int} = intrinsic rat hepatic clearance.

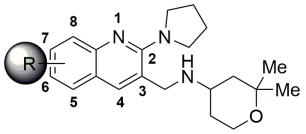
analogues. However these modifications did not show a positive effect on antiparasitic activity.

We next turned our attention to the substituent at the 2-position of the quinoline core (Table II). We observed that removal or replacement of the pyrrolidine ring was unsuccessful with the exception of piperidine **31** that led to an 8-fold decrease in activity. Rings with two heteroatoms, small acyclic amines or even hydrogen substantially improved solubility (compounds **34, 37, 39, 43, 49**). Although the pyrrolidine ring was essential for potency, the initial hit **12** with this fragment showed the higher clearance by human microsomes. Thus, all other substituents tested, afforded better stability particularly the unsubstituted derivative **39** which displayed by far the lowest intrinsic clearance for this sub-series, probably because its lower logD.

Then we focused on the effect of the 8-methyl substituent on antiparasitic activity and *in vitro* clearance by microsomes and hepatocytes (Table III). Replacement by hydrogen (derivative **53**) led to a 60-fold drop in potency and moving the methyl group around the aromatic ring reduced activity even further (analogues **57, 61**). However, it was possible to replace the methyl group with a chlorine (quinoline **65**) and retain activity, but without improving the remaining properties. In compound **66**, a hydroxyl function was installed, which significantly lowered logD and improved solubility and metabolic stability while only reducing activity 12-fold.

To ensure the series maintained activity against parasites resistant to historical antimalarial drugs, the most active compounds, the original hit

TABLE III
SAR on the left hand-side.



R	IC_{50} NF54 (nM)	$logD_{7.4}$	Solub. (μM)	H Mics CL_{int} (μl/min/mg)	Rat Heps CL_{int} (μl/min/10^6)
8-Me **12**	22	3.8	29	102	>300
8-H **53**	1482	2.9	660	55	>300
7-Me **57**	8761	3.0	702	66	>300
6-Me **61**	5220	3.1	686	75	285
8-Cl **65**	24	3.6	27	138	275
8-OH **66**	270	2.5	362	43	141

Solub = solubility in PBS; H Mics CL_{int} = intrinsic human microsomal clearance; Rat Heps CL_{int} = intrinsic rat hepatic clearance.

12 and **65** were tested against eight drug-resistant *Pf* strains (Table IV).

We observed that activity did not decrease substantially against most strains, except for an aproximate 8-fold reduction against K1 for both compounds. These results indicated a concerning level of resistance for this series which led us to determine activity against K1 for the most potent compounds (Table V).

In the examples described in Table V, we observed the same trend as for quinolines **12** and **65** with an 8-fold reduction in activity suggesting that resistance is inherent in this series which would be a barrier to further development.

CONCLUSIONS

After our systematic SAR analysis we were able to prepare the chlorinated analogue **65**, with comparable potency (IC_{50} = 24 nM) to the hit **12**. A thorough investigation of physicochemical and DMPK properties revealed low metabolic stability in human microsomes and rat hepatocytes as additional issue with this series.

The quinoline heterocycle is an useful platform to find active drug candidates against the malaria parasite. Although very potent candidates can be found with this scaffold as observed in the history of antimalarial drugs (quinine, chloroquine and mefloquine for example) it is important also to verify the resistance levels on different *Pf* strains as the appearance of this effect is frequently observed. The series appears to have a different mode of action to other quinoline drugs, such as chloroquine and mefloquine, as illustrated by the series showing a slower rate of killing in the blood stage. Further studies to identify the mode of action are planned.

ACKNOWLEDGMENTS

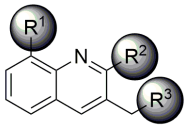
We especially thank Prof. Marcos Eberlin (Thomsom Laboratory) for HRMS analyses. We are grateful to Sergio Wittlin, Sibylle Sax and

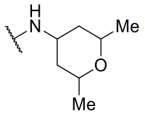
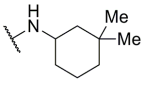
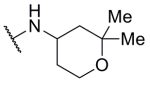
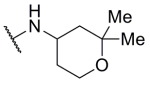
TABLE IV
Resistance profile for compounds 12 and 65.

Pf strain	Derivative **12** IC_{50} (nM)	Derivative **12** IC_{50} (relative to NF54)	Derivative **65** IC_{50} (nM)	Derivative **65** IC_{50} (relative to NF54)	Resistance against selected drugs
NF54	22	1	24	1	
D6	15	0.7	20	0.8	
HB3	44	2	34	1.4	CYC, PM
7G8	46	2.1	34	1.4	(CQ)[a], CYC, PM
FCB	52	2.4	53	2.2	CQ
Dd2	69	3.2	40	1.7	CQ, CYC, PM
TM90C2B	81	3.7	75	3.1	AT, CQ, CYC, PM
V1/S	87	4.0	90	3.8	CQ, CYC, PM
K1	173	7.9	200	8.3	CQ, CYC, PM

AT = atovaquone; CQ = chloroquine; CYC = cycloguanil; PM = pyrimethamine; [a] close to resistance threshold.

TABLE V
Activity on Pf K1 strain of selected compounds.



compound	R^1	R^2	R^3	IC_{50} *Pf* K1 (nM)	IC_{50} (relative to NF54)
16	Me			498	6.7
21	Me			1374	6.0
31	Me			1640	9.8
66	OH			2302	8.5

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ratio studies. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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