Synthesis and evaluation of antimalarial properties of novel 4-aminoquinoline hybrid compounds

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Abstract

Pharmacophore hybridization has recently been employed in the search for antimalarial lead compounds. This approach chemically links two pharmacophores, each with their own antimalarial activity and ideally with different modes of action, into a single hybrid molecule with the goal to improve therapeutic properties. In this paper we report the synthesis of novel 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds. The chlorinated 4-aminoquinoline scaffold is the core structure of chloroquine, an established antimalarial drug, while the primary sulfonamide functional group has a proven track record of efficacy and safety in many clinically used drugs and was recently shown to exhibit some antimalarial activity. The activity of the hybrid compounds was determined against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *Plasmodium falciparum* strains. While the hybrid compounds had lower antimalarial activity when compared to chloroquine, they demonstrated a number of interesting structure-activity relationship (SAR) trends including the potential to overcome the resistance profile of chloroquine.

1 Introduction

Malaria remains a major global health issue that affects around 3.2 billion people and results in ~1 million deaths annually(1). Efforts to control and eradicate malaria are hindered by the absence of a suitable vaccine and increasing resistance to clinically used drugs(2-7). To address the need for safe and effective new antimalarial drugs that overcome issues of cross-resistance, numerous antimalarial drug discovery approaches are being actively pursued. These include whole-cell and target-based screening approaches, generation of pharmacokinetically optimized analogues of existing drugs, development of combination therapies, investigation of drug resistance reversers, and generation of hybrid compounds(8). In the latter approach two antimalarial pharmacophores, preferably with different modes of action, are chemically combined. The aim is to generate hybrid compounds that have improved antimalarial activity,

stability, and/or solubility over the separate pharmacophores(9). There are a number of potential advantages to the hybrid arrangement over combination therapies. A single agent ensures the same pharmacokinetics and avoids additional stress on clearance mechanisms that may accompany the administration of multiple agents. A number of antimalarial hybrids currently under investigation have focused on the antimalarial drug chloroquine, comprising a chlorinated 4-aminoquinoline scaffold, Figure 1(10-17). Chloroquine accumulates in the food vacuole of the parasite and acts by interfering with the polymerisation of toxic heme moieties, the by-products of haemoglobin digestion in the intraerythrocytic cycle of the parasite(18-21). Although *Plasmodium falciparum*, the most lethal human malaria parasite species, has developed resistance to chloroquine in most areas of the world, this drug remains effective for the treatment of other malaria parasite species such as *Plasmodium vivax*(8, 22). In addition chloroquine is inexpensive, easily administered, and safe for use in infants and pregnancy. These favourable biopharmaceutical properties make strategies aimed at overcoming issues of parasite resistance to this drug attractive. Hybrid compounds that retain the effective antimalarial attributes of chloroquine whilst overcoming the primary limitation (i.e. drug resistance) may therefore have potential therapeutic benefit. Recent examples that employ pharmacophore hybridization as an approach to antimalarial drug discovery include chloroquine-triazine hybrids,(23-25) chloroquine-pyrimidine hybrids,(26) trioxaquinines(10, 27, 28), the mefloquine-artesunate hybrid MEFAS(29), the ferrocene-chloroquine hybrid, ferroquine (11, 12, 30) and others(14-16, 31, 32), Figure 1. The majority of these compounds have shown improved antimalarial activity over the parent compound when tested against drug resistant parasites(10-13, 15, 16, 27-29, 31, 32). Furthermore several of these hybrid compounds have also shown comparable or better *in vivo* efficacy when tested in mouse malaria models(10-12, 27-29, 32). Three hybrid compounds have advanced further along the malaria drug discovery pipeline with the mefloquine-artesunate hybrid MEFAS and the

trioxaquine PA11103/SAR116242 currently undergoing pre-clinical development, while the organometallic drug candidate ferroquine is in phase II clinical trials(11, 28, 29).

Figure 1. The antimalarial drug chloroquine and representative hybrid antimalarial compounds.

Recently we reported the *in vitro* antimalarial activity of a series of primary sulfonamides. These compounds had IC_{50} s between 0.9 - 4 μ M and good selectivity for *P. falciparum* versus mammalian cells (selectivity index (SI) >50-100).(33) The IC_{50} s of the most potent and selective of these compounds did not differ significantly between chloroquine-sensitive (3D7) and chloroquine resistant (Dd2) *P. falciparum* lines, indicating a different mode of action to chloroquine. It is widely reported that primary sulfonamides (R-SO₂NH₂) are excellent inhibitors of carbonic anhydrase (CA) zinc metalloenzymes(34). There is substantial interest in disrupting CA activity in pathogens, including pathogenic bacteria (*Brucella* spp., *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Salmonella enterica* serovar Typhimurium, *Vibrio cholerae* and *Helicobacter pylori*), pathogenic fungi (*Candida albicans*, *C. glabrata*, and *Cryptococcus neoformans*) and more recently the protozoan parasite

Trypanosoma cruzi(35-41). While the antimalarial target of these compounds is still under investigation in our lab, *PfCA* is a possible candidate. Given the early findings with primary sulfonamides and that hybrid drugs of chloroquine should ideally have an independent target to offset parasite resistance to chloroquine, here we investigate the combination 7-chloro-4-aminoquinolines and primary benzene sulfonamide hybrid compounds for altered antimalarial activity. In this study we have evaluated the *in vitro* antimalarial activity of several 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds alongside analogues lacking a primary sulfonamide moiety as controls.

2 Methods and Materials

2.1 Chemistry General.

All starting materials and reagents were purchased from commercial suppliers. 3-Azidopropyl-1-amine hydrochloride(42) , 4-ethynylbenzenesulfonamide(43), 3-ethynylbenzenesulfonamide(44) 4-azidobenzenesulfonamide(45) and 3-azidobenzenesulfonamide(46) were synthesized as described earlier. All reactions were monitored by TLC. TLC plates were visualized with UV light and ninhydrin stain (1 g of ninhydrin in 100 mL of EtOH containing 3% (v/v) acetic acid). Silica gel flash chromatography was performed using silica gel 60 Å (230-400 mesh). ¹H NMR were acquired at 500 MHz and ¹³C NMR at 125 MHz at 30 °C. For ¹H and ¹³C NMR acquired in CDCl₃ chemical shifts (δ) are reported in ppm relative to the solvent residual peak: proton (δ 7.27 ppm) and carbon (δ 77.2 ppm). Chemical shifts for ¹H and ¹³C NMR acquired in DMSO-*d*₆ are reported in ppm relative to residual solvent proton (δ 2.50 ppm) and carbon (δ 39.5 ppm) signals, respectively. Assignments for ¹H NMR were confirmed by ¹H-¹H gCOSY, while assignments for ¹³C NMR were confirmed by ¹H-¹³C HSQC. Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); q (quintet); m (multiplet); dd (doublet of doublet);

ddd (doublet of doublet of doublet); br (broad). Coupling constants are reported in Hertz (Hz). Melting points are uncorrected. High and low resolution electrospray ionization mass spectra were acquired using electrospray as the ionization technique in positive ion and/or negative ion modes as stated. All MS analysis samples were prepared as solutions in MeOH. Purity of all compounds was $\geq 95\%$ by NMR and HPLC.

2.2 Chemistry Synthesis.

General Procedure 1. Synthesis of hybrid and control compounds 4-12 using CuAAC.

A mixture of azide (1.0 equiv.) and alkyne (1.0 equiv.) was suspended in H₂O/*t*BuOH 1:1 (2-4 mL). A freshly prepared solution of sodium ascorbate (0.4 equiv.) and CuSO₄·5H₂O (0.2 equiv) in water (1 mL) was added. The resulting bright yellow suspension was stirred vigorously at 40 °C until no further change was observed (TLC). The reaction mixture was diluted with 10 mL of water, cooled in an ice bath, filtered, washed with water (2 × 3 mL) and dried in vacuo. To remove copper ions the crude product was dissolved in DMSO (2-3 mL) and EDTA (30-80 mg, 0.8-1.0 equiv.) was added. The solution was sonicated for 10 min, filtered and washed with DMSO (1 mL). The filtrate was diluted with water (5-8 mL) and cooled in an ice bath, the precipitate that formed was filtered and washed with water (3 mL) and EtOAc (2 × 3 mL). Further purification was carried out as described below.

***N*-(Prop-2-yn-1-yl)-7-chloroquinolin-4-amine (1).** 4,7-Dichloroquinoline (0.200 g, 1.00 mmol, 1 equiv.) and propargylamine (0.26 mL, 4.00 mmol, 4 equiv.) were combined under N₂. The 100 mL reaction flask was sealed and heated for 17 h at 110 °C. The reaction mixture was diluted with MeOH (10 mL), transferred to a round bottom flask and concentrated under reduced pressure. The crude product was dissolved in EtOAc (50 mL), washed with water (3 × 50 mL), and the aqueous fractions combined and extracted with EtOAc (2 × 50 mL). The organic fractions were combined, dried over MgSO₄, filtered and concentrated under reduced

pressure. The residue was purified by column chromatography (EtOAc/Hexanes, 4:6) to obtain **1** (0.147 g, 72%) as a pale yellow solid. $R_f = 0.44$ (EtOAc). mp 158 °C (dec.). ^1H NMR (500 MHz, DMSO- d_6): δ 3.18 (t, $J = 1.8$ Hz, 1H, $\equiv\text{CH}$), 4.14 (dd, $J = 5.5, 2.0$ Hz, 2H, CH_2), 6.60 (d, $J = 5.5$ Hz, 1H, H_3), 7.49 (dd, $J = 8.8, 1.8$ Hz, 1H, H_6), 7.47 (t, $J = 5.5$ Hz, 1H, NH), 7.84 (d, $J = 2.0$ Hz, 1H, H_8), 8.20 (d, $J = 9.0$ Hz, 1H, H_5), 8.49 (d, $J = 4.5$ Hz, 1H, H_2). Assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6): δ 32.09 (NHCH $_2$), 74.2 (C $\equiv$), 80.9 ($\equiv\text{CH}$), 100.3 (C $_3$), 118.1 (C), 124.6 (C $_5$), 125.0 (C $_6$), 128.1 (C $_8$), 134.0 (C), 149.4 (C), 149.7 (C), 152.3 (C $_2$). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI $^+$): $m/z = 217.1, 219.1$ [M+H; $^{35}\text{Cl}, ^{37}\text{Cl}$] $^+$. HRMS (ESI) calcd for C $_{12}\text{H}_{10}\text{ClN}_2$ [M+H] $^+$ 217.0527, found 217.0528.

***N*-(But-3-yn-1-yl)-7-chloroquinolin-4-amine (2).** 4,7-Dichloroquinoline (0.200 g, 1.00 mmol, 1 equiv.), 1-amino-3-butyne (0.140 mg, 2.00 mmol, 2 equiv.) and Et $_3\text{N}$ (0.28 mL, 2.00 mmol, 2 equiv.) were combined under N $_2$. The 100 mL reaction flask was sealed and heated for 63 h at 110 °C. The reaction mixture was cooled, diluted with MeOH (40 mL), transferred to a round bottom flask and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc/Hexanes, 1:1) to obtain **2** (0.092 g, 40%) as white solid. $R_f = 0.36$ (EtOAc). mp 164 °C (dec.). ^1H NMR (500 MHz, DMSO- d_6): δ 2.56 (m, 2H, CH $_2\text{C}\equiv$), 2.87 (t, $J = 2.5$ Hz, 1H, $\equiv\text{CH}$), 3.46 (q, $J = 6.5$ Hz, 2H, NHCH $_2$), 6.53 (d, $J = 5.5$ Hz, 1H, H_3), 7.42 (t, $J = 5.3$ Hz, 1H, NH), 7.47 (dd, $J = 9.0, 2.0$ Hz, 1H, H_6), 7.80 (d, $J = 2.0$ Hz, 1H, H_8), 8.24 (d, $J = 9$ Hz, 1H, H_5), 8.42 (d, $J = 5.5$ Hz, H_2). ^{13}C NMR (125 MHz, DMSO- d_6): δ 18.2 (CH $_2\text{C}\equiv$), 41.8 (NHCH $_2$), 72.8 (C $\equiv$), 82.7 ($\equiv\text{CH}$), 99.3 (C $_3$), 117.9 (C), 124.4 (C $_5$), 124.7 (C $_6$), 128.0 (C $_8$), 133.9 (C), 149.5 (C), 150.2 (C), 152.4 (C $_2$). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI $^+$): $m/z = 231.1, 233.1$ [M+H; $^{35}\text{Cl}, ^{37}\text{Cl}$] $^+$. HRMS (ESI) calcd for C $_{13}\text{H}_{12}\text{ClN}_2$ [M+H] $^+$ 231.0684, found 231.0685.

***N*-(3-Azidopropyl)-7-chloroquinolin-4-amine (3).** 4,7-Dichloroquinoline (0.582 g, 2.34 mmol, 1 equiv.), 3-azidopropyl-1-amine (hydrochloride salt) (0.800 g, 5.88 mmol, 2.5 equiv.), and Et₃N (1.64 mL, 11.8 mmol, 5 equiv.) were combined under N₂. The 100 mL reaction flask was sealed and heated for 24 h at 110 °C. The reaction mixture was cooled, diluted with MeOH (40 mL), transferred to a round bottom flask and concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), washed with NaOH (5.0 M, 40 mL) and the aqueous fraction extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc/hexanes, 55:45) to obtain **3** (0.605 g, 79%) as pale yellow solid. *R_f* = 0.25 (EtOAc). mp 144 °C (dec.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.93 (q, *J* = 6.8 Hz, 2H, CH₂), 3.35 (t, *J* = 6.3 Hz, 2H, NHCH₂), 3.50 (t, *J* = 6.8 Hz, 2H, CH₂N₃), 6.49 (d, *J* = 5.5 Hz, 1H, H₃), 7.30 (t, *J* = 5.3 Hz, 1H, NH), 7.45 (dd, *J* = 9.0 and 2.5 Hz, 1H, H₆), 7.80 (d, *J* = 2.0 Hz, 1H, H₈), 8.27 (d, *J* = 9.0 Hz, 1H, H₅), 8.41 (d, *J* = 5.5 Hz, 1H, H₂). Assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 27.6 (CH₂), 40.1 (NHCH₂), 49.1 (CH₂N₃), 99.2 (C₃), 118.0 (C), 124.5 (C₅ and ₆), 128.0 (C₈), 133.9 (C), 149.6 (C), 150.5 (C), 152.4 (C₂). Assignments were confirmed by ¹H-¹³C gHSQC. LRMS (ESI⁺): *m/z* = 262.1, 264.1 [M+H; ³⁵Cl, ³⁷Cl]⁺. HRMS (ESI) calcd for C₁₂H₁₂ClN₅ [M+H]⁺ 262.0853, found 262.086.

7-Chloro-*N*-((1-(4-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (4). The title compound **4** was synthesized from alkyne **1** (0.040 g, 0.185 mmol, 1 equiv.) and 4-azidobenzenesulfonamide (0.037 g, 0.185 mmol, 1 equiv.) according to general procedure 1 in 22 h. The residue after EDTA treatment was dried in vacuo to obtain **4** (0.006 g, 8%) as pale pink solid. *R_f* = 0.08 (EtOAc). mp > 250 °C (dec.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 4.71 (d, *J* = 4.0 Hz, 2H, CH₂), 6.66 (d, *J* = 4.0 Hz, 1H, H₃), 7.521-7.503 (m, 3H, SO₂NH₂ and H₆), 7.83 (s, 1H, H₈), 8.00 (d, *J* = 8.5 Hz, 2H, H_{3',5'}), 8.08 (s, 1H, NH), 8.12 (d, *J* = 8.0 Hz, 2H, H_{2',6'}), 8.34 (d, *J* = 8.5 Hz, H₅), 8.44 (s, 1H, H₂), 8.88 (s, 1H, H_{triazole}). Assignments were confirmed by ¹H-

^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6): δ 38.4 (CH_2), 99.8 (C_3), 117.8 (C_{4a}), 120.7 ($\text{C}_{3',5'}$), 122.1 ($\text{CH}_{\text{triazole}}$), 124.7 (C_5), 125.0 (C_6), 127.5 (C_8), 127.9, ($\text{C}_{2',6'}$), 134.2 (C_7), 139.0 ($\text{C}_{4'}$), 144.2 ($\text{C}_{1'}$), 146.2 ($\text{C}_{\text{triazole}}$), 148.9 (C_{8a}), 150.5 (C_4), 151.8 (C_2). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI $^+$): m/z = 415.1, 417.0 [$\text{M}+\text{H}$; ^{35}Cl , ^{37}Cl] $^+$. HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{16}\text{ClN}_6\text{O}_2\text{S}$ [$\text{M}+\text{H}$] $^+$ 415.0738, found 415.0744.

7-Chloro-*N*-((1-(3-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)quinolin-4-amine

(**5**). The title compound **5** was synthesized from alkyne **1** (0.040 g, 0.185 mmol, 1 equiv.) and 3-azidobenzenesulfonamide (0.037 g, 0.185 mmol, 1 equiv.) according to general procedure 1 in 19 h. Purification of crude product by flash chromatography (EtOAc) afforded **5** (0.014 g, 40%) of as white solid. R_f = 0.08 (EtOAc). mp > 250 °C (dec.). ^1H NMR (500 MHz, DMSO- d_6): δ 4.70 (d, J = 4.5 Hz, 2H, CH_2), 6.63 (d, J = 5.0 Hz, 1H, H_3), 7.49 (d, J = 8.5 Hz, 1H, H_6), 7.55 (s, 2H, SO_2NH_2), 7.79 (t, J = 8.0 Hz, $\text{H}_{5'}$), 7.82 (s, 1H, H_8), 7.90 (d, J = 7.0 Hz, 1H, $\text{H}_{6'}$ or $4'$), 7.96 (t, J = 4.5 Hz, 1H, NH), 8.12 (d, J = 7.5 Hz, 1H, $\text{H}_{6'}$ or $4'$), 8.32 (d, J = 8.5 Hz, 1H, H_5), 8.36 (s, 1H, $\text{H}_{2'}$), 8.43 (s, 1H, H_2), 8.87 (s, 1H, $\text{H}_{\text{triazole}}$). Assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6): δ 38.4 (CH_2), 99.8 (C_3), 117.5 (C_{4a}), 121.9 ($\text{C}_{2'}$) 122.1 ($\text{CH}_{\text{triazole}}$), 123.4 ($\text{C}_{4'}$ or $6'$), 124.6 (C_5), 124.8 (C_6), 125.8 ($\text{C}_{6'}$ or $4'$), 128.0 (C_8), 131.3 ($\text{C}_{5'}$), 133.9 (C_7), 137.2 (C), 146.2 (C), 146.3 ($\text{C}_{\text{triazole}}$), 149.6 (C_{8a}), 150.2 (C_4) 152.4 (C_2). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI $^+$): m/z = 415.1, 417.1 [$\text{M}+\text{H}$; ^{35}Cl , ^{37}Cl] $^+$. HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{16}\text{ClN}_6\text{O}_2\text{S}$ [$\text{M}+\text{H}$] $^+$ 415.0738, found 415.0744.

7-Chloro-*N*-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (**6**). The title compound **6** was synthesized from alkyne **1** (0.035 g, 0.162 mmol, 1 equiv.) and phenylazide (0.022 g, 0.185 mmol, 1 equiv.) according to general procedure 1 in 21 h. The residue after EDTA treatment was dried in vacuo to obtain **6** (0.015 g, 28%) as pale pink solid. R_f = 0.2 (EtOAc). mp = 171 °C (dec.). ^1H NMR (500 MHz, DMSO- d_6): δ 4.68 (d, J = 4 Hz, 2H, CH_2),

6.64 (d, $J = 3.5$ Hz, 1H, H₃), 7.49 (m, 2H, H₆ and H_{4'}), 7.79 (t, $J = 6.8$ Hz, 2H, H_{3',5'}), 7.82 (s, 1H, H₈), 7.89 (dd, $J = 0.8, 7.3$ Hz, 2H, H_{2',6'}), 7.97 (s, 1H, NH), 8.33 (d, $J = 8.5$ Hz, 1H, H₅), 8.43 (s, 1H, H₂), 8.78 (s, 1H, H_{triazole}). Assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 38.4 (CH₂), 120.4 (C_{2',6'}), 121.9 (CH_{triazole}), 124.7 (C₅), 124.8 (C₆), 128.0 (C₈), 129.0 (C_{4'}), 130.3 (C_{3',5'}), 133.9 (C₇), 137.1 (C_{1'}), 145.8 (C_{triazole}), 150.2 (C₄) 152.3 (C₂). Assignments were confirmed by ¹H-¹³C gHSQC. LRMS (ESI⁺): $m/z = 336.1, 338.1$ [M+H; ³⁵Cl, ³⁷Cl]⁺. HRMS (ESI) calcd for C₁₈H₁₅ClN₅ [M+H]⁺ 336.1010, found 336.1012.

7-Chloro-*N*-(2-(1-(4-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)ethyl)quinolin-4-amine

(**7**). The title compound **7** was synthesized from alkyne **2** (0.040 g, 0.174 mmol, 1 equiv.) and 4-azidobenzenesulfonamide (0.034 g, 0.174 mmol, 1 equiv.) according to general procedure 1 in 19 h. Purification of crude product by flash chromatography (MeOH/CH₂Cl₂, 1:9) afforded **7** (0.012 g, 16%) as white solid. $R_f = 0.25$ (MeOH/CH₂Cl₂, 1:9). mp > 250 °C (dec.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 3.14 (t, $J = 6.0$ Hz, 2H, CH₂), 3.67 (q, $J = 6.5$ Hz, 2H, NHCH₂), 6.60 (d, $J = 4.0$ Hz, 1H, H₃), 7.46 (d, $J = 9.0$ Hz, 1H, H₆), 7.51 (s, 3H, SO₂NH₂ and NH), 7.81 (s, 1H, H₈), 8.03 (d, $J = 7.5$ Hz, 2H, H_{2',6'} or 3',5'), 8.09 (d, $J = 8.0$ Hz, 2H, H_{3',5'} or 2',6'), 8.26 (d, $J = 9.0$ Hz, 1H, H₅), 8.45 (s, 1H, H₂), 8.80 (s, 1H, H_{triazole}). Assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 24.7 (CH₂), 42.4 (NHCH₂), 120.5 (CH, C_{3',5'}), 121.6 (CH_{triazole}), 124.6 (C₅), 124.7 (C₆), 128.0 (C_{2',6'}), 133.9 (C₇), 139.2 (C_{4'}), 144.1 (C_{1'}), 146.4 (C_{triazole}), 150.3 (C₄). Assignments were confirmed by ¹H-¹³C gHSQC. LRMS (ESI⁺): $m/z = 429.1, 431.0$ [M+H; ³⁵Cl, ³⁷Cl]⁺. HRMS (ESI) calcd for C₁₉H₁₈ClN₆O₂S [M+H]⁺ 429.0895, found 429.0901.

7-Chloro-*N*-(2-(1-(3-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)ethyl)quinolin-4-amine

(**8**). The title compound **8** was synthesized from alkyne **2** (0.040 g, 0.174 mmol, 1 equiv.) and 3-azidobenzenesulfonamide (0.034 g, 0.174 mmol, 1 equiv.) according to general procedure 1

in 19 h. Purification of crude product by flash chromatography (MeOH/CH₂Cl₂, 1:9) afforded **8** (0.030 g, 41%) as yellow solid. R_f = 0.29 (MeOH/CH₂Cl₂, 1:9). mp > 250 °C (dec.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 3.15 (t, J = 7.3 Hz, 2H, CH₂), 3.68 (q, J = 6.7 Hz, 2H, NHCH₂), 6.62 (d, J = 5.5 Hz, 1H, H₃), 7.47 (dd, J = 1.8 and 8.8 Hz, 1H, H₆), 7.58 (s, 3H, SO₂NH₂ and NH), 7.83-7.80 (m, 2H, H_{5'} and H_{2'}), 7.92 (d, J = 7.5 Hz, 1H, H_{4'} or 6'), 8.09 (dd, J = 1.0 and 8.0 Hz, 1H, H_{6'} or 4'), 8.27 (d, J = 9.0 Hz, 1H, H₅), 8.36 (t, J = 2.0 Hz, 1H, H₈), 8.45 (d, J = 4.5 Hz, 1H, H₂), 8.80 (s, 1H, H_{triazole}). Assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 24.8 (CH₂), 42.5 (NHCH₂), 99.3 (C₃), 117.4 (C_{4a}), 117.9 (C), 121.6 (CH_{triazole}), 123.3 (CH, C_{4'} or 6'), 124.6 (C₅), 124.7 (C₆), 125.8 (C_{6'} or 4'), 127.7 (C_{5'} or 2'), 127.7 (C₈), 131.4 (C_{2'} or 5'), 134.1 (C₇), 137.3 (C_{1'} or 3'), 146.3 (C_{1'} or 3'), 146.4 (C_{triazole}), 149.2 (C_{1a}), 150.6 (C₄), 152.1 (C₂). Assignments were confirmed by ¹H-¹³C gHSQC. LRMS (ESI⁺): m/z = 429.1, 431.1 [M+H; ³⁵Cl, ³⁷Cl]⁺. HRMS (ESI) calcd for C₁₉H₁₈ClN₆O₂S [M+H]⁺ 429.0895, found 429.0902.

7-Chloro-N-(2-(1-phenyl-1*H*-1,2,3-triazol-4-yl)ethyl)quinolin-4-amine (9). The title compound **9** was synthesized from alkyne **2** (0.035 g, 0.152 mmol, 1 equiv.) and phenylazide (0.021 g, 0.174 mmol, 1.1 equiv.) according to general procedure 1 in 26 h. Purification of crude product by flash chromatography (MeOH/CH₂Cl₂, 1:9) afforded **9** (0.010 g, 19%) as white solid. R_f = 0.30 (MeOH/CH₂Cl₂, 1:9). mp > 246 °C (dec.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 3.13 (t, J = 7.5 Hz, 2H, CH₂), 3.67 (q, J = 6.7 Hz, 2H NHCH₂), 6.61 (d, J = 5.5 Hz, 1H, H₃), 7.47 (dd, J = 1.5, 8.5 Hz, 1H, H₆), 7.49 (d, J = 7.5 Hz, 1H, H_{4'}), 7.52 (t, J = 5.5 Hz, 1H, NH), 7.60 (T, J = 7.8 Hz, 2H, H_{3',5'}), 7.81 (s, 1H, H₈), 7.87 (d, J = 8 Hz, 2H, H_{2',6'}), 8.26 (d, J = 9 Hz, 1H, H₅), 8.45 (s, 1H, H₂), 8.80 (s, 1H, H_{triazole}). Assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆): δ 24.8 (CH₂), 42.5 (NHCH₂), 99.3 (C₃), 120.3 (C_{2',6'}), 121.4 (CH_{triazole}), 124.5 (C₅), 124.6 (C₆), 128.0 (C₈), 128.9 (C_{4'}), 130.4 (C_{3',5'}), 133.9 (C₇), 137.2 (C_{1'}), 146.0 (C_{triazole}), 150.3 (C₄) 152.4 (C₂). Assignments were confirmed by ¹H-

^{13}C gHSQC. LRMS (ESI⁺): m/z = 350.1, 352.3 [M+H; ^{35}Cl , ^{37}Cl]⁺. HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{17}\text{ClN}_5$ [M+H]⁺ 350.1167, found 350.1170.

7-Chloro-*N*-(3-(1-(4-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)propyl)quinolin-4-amine

(**10**). The title compound **10** was synthesized from azide **3** (0.086 g, 0.33 mmol, 1 equiv.) and 4-ethynylbenzenesulfonamide (0.070 g, 0.39 mmol, 1 equiv.) according to general procedure 1 in 15 h. This compound was not treated with EDTA. The crude product was dissolved in hot MeOH (70 mL) and the solvent volume reduced to 10 mL. On cooling a precipitate formed, this was filtered, washed with cold MeOH (5 mL) and purified by flash chromatography (MeOH/CH₂Cl₂, 1:9) to afford **10** (0.041 g, 63%) as white solid. R_f = 0.63 (MeOH/CH₂Cl₂, 1:9). mp > 250 °C (dec.). ^1H NMR (500 MHz, DMSO- d_6): δ 2.31 (m, J = 6.9 Hz, 2H, CH₂), 3.37 (q, J = 6.3 Hz, 2H, NHCH₂), 4.60 (t, J = 7.0 Hz, 2H, CH₂N), 6.49 (d, J = 5.5 Hz, 1H, H₃), 7.38 (s, 3H, SO₂NH₂ and NH), 7.46 (dd, J = 9.0 and 2.0 Hz, 1H, H₆), 7.80 (d, J = 1.5 Hz, 1H, H₈), 7.90 (d, J = 8.0 Hz, 2H, H_{2',6'}), 8.02 (d, J = 8.0 Hz, 2H, H_{3',5'}), 8.27 (d, J = 9.0 Hz, 1H, H₅), 8.41 (d, J = 5.0 Hz, 1H, H₂), 8.75 (s, 1H, H_{triazole}). Assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6): δ 28.8 (CH₂), 40.4 (NHCH₂), 48.2 (CH₂N), 99.3 (C₃), 117.9 (C_{4a}), 123.1 (CH_{triazole}), 124.5 (C₆ or 5), 124.6 (C₅ or 6), 125.8 (C_{3',5'}), 126.8 (C_{2',6'}), 127.9 (C₈), 133.9 (C₇), 134.4 (C_{4'}), 143.6 (C_{1'}), 145.6 (C_{triazole}), 149.4 (C_{8a}), 150.5 (C₄), 152.3 (C₂). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI⁺): m/z = 443.1, 445.1 [M+H; ^{35}Cl , ^{37}Cl]⁺. HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{20}\text{ClN}_6\text{O}_2\text{S}$ [M+H]⁺ 443.1051, found 443.1058.

7-Chloro-*N*-(3-(1-(3-aminosulfonylphenyl)-1*H*-1,2,3-triazol-4-yl)propyl)quinolin-4-amine

(**11**). The title compound **11** was synthesized from azide **3** (0.078 g, 0.30 mmol, 1 equiv.) and 3-ethynylbenzenesulfonamide (0.064 g, 0.36 mmol, 1.2 equiv.) according to general procedure 1 in 64 h. After EDTA treatment the residue was dried in vacuo to obtain **11** (0.018 g, 13%) as white solid. R_f = 0.13 (MeOH/CH₂Cl₂, 1:9). mp > 250 °C (dec.). ^1H NMR (500 MHz, DMSO-

d_6): δ 2.32 (m, $J = 7$ Hz, 2H, CH_2), 2.36 (q, $J = 6.2$ Hz, 2H, NHCH_2), 4.60 (t, $J = 6.8$ Hz, 2H, CH_2N), 6.49 (d, $J = 5.0$ Hz, 1H, H_3), 7.37 (t, $J = 5.0$ Hz, 1H, NH), 7.43 (s, 2H, SO_2NH_2), 7.46 (d, $J = 9.0$ Hz, 1H, H_6), 7.65 (t, $J = 7.8$ Hz, 1H, $\text{H}_{5'}$), 7.78 (d, $J = 0.5$ Hz, 1H, $\text{H}_{4'}$), 7.79 (s, 1H, $\text{H}_{2'}$), 8.01 (d, $J = 8$ Hz, 1H, $\text{H}_{6'}$), 8.27 (d, $J = 9$ Hz, 1H, H_5), 8.34 (s, 1H, H_8), 8.41 (s, 1H, H_2), 8.75 (s, 1H, $\text{H}_{\text{triazole}}$). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 28.8 (CH_2N), 40.0 (CH_2), 48.2 (NHCH_2), 99.3 (C_3), 118.0 (C_{4a}), 122.5 ($\text{C}_{2'}$), 122.7 ($\text{CH}_{\text{triazole}}$), 124.5 (C_5), 124.6 (C_6), 125.3 ($\text{C}_{4'}$), 128.0 (C_8), 128.7 ($\text{C}_{6'}$), 130.1 ($\text{C}_{5'}$), 132.0 ($\text{C}_{3'}$), 133.9 (C_7), 145.4 ($\text{C}_{1'}$), 145.6 ($\text{C}_{\text{triazole}}$), 149.5 (C_{1a}), 150.4 (C_4), 152.4 (C_2). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI^+): $m/z = 443.1, 445.1$ [$\text{M}+\text{H}; ^{35}\text{Cl}, ^{37}\text{Cl}$] $^+$. HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{20}\text{ClN}_6\text{O}_2\text{S}$ [$\text{M}+\text{H}$] $^+$ 443.1051, found 443.1058.

7-Chloro-*N*-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine (12). The title compound **12** was synthesized from azide **3** (0.130 g, 0.5 mmol, 1 equiv.) and phenylacetylene (0.096 g, 0.55 mmol, 1.1 equiv.) according to general procedure 1 in 67 h. This compound was not treated with EDTA. Purification of crude product by flash chromatography ($\text{MeOH}/\text{CH}_2\text{Cl}_2$, 1:9) afforded **12** (0.080 g, 44%) as white solid. $R_f = 0.32$ ($\text{MeOH}/\text{CH}_2\text{Cl}_2$, 1:9). mp > 250 °C (dec.). ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 2.31 (q, $J = 6.8$ Hz, 2H, CH_2), 3.36 (q, $J = 6.2$ Hz, 2H, NHCH_2), 4.57 (t, $J = 7$ Hz, 2H, CH_2), 6.49 (d, $J = 5.5$ Hz, 2H, H_3), 7.34 (t, $J = 7.3$ Hz, 1H, $\text{H}_{4'}$), 7.50 (t, $J = 7.8$ Hz, 1H, NH), 7.47-7.43 (m, 3H, H_6 and $\text{H}_{2',6'}$), 7.80 (d, $J = 2$ Hz, 1H, H_8), 7.84 (d, $J = 8$ Hz, 2H, $\text{H}_{3',5'}$), 8.27 (d, $J = 9$ Hz, 1H, H_5), 8.41 (d, $J = 4$ Hz, 1H, H_2), 8.61 (s, 1H, $\text{H}_{\text{triazole}}$). Assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 28.8 (NHCH_2), 40.1 (CH_2), 48.1 (CH_2N), 122.0 ($\text{CH}_{\text{triazole}}$), 124.6 (C_6 and C_5), 125.6 ($\text{C}_{2',6'}$ and C_3), 127.9 (C), 128.3 ($\text{C}_{4'}$), 129.3 (C_8 and $\text{C}_{3',5'}$), 131.3 (C), 133.9 (C_7), 146.8 ($\text{CH}_{\text{triazole}}$), 149.4 (C), 150.4 (C_4), 152.1 (C_2). Assignments were confirmed by ^1H - ^{13}C gHSQC. LRMS (ESI^+): $m/z = 364.1, 366.1$ [$\text{M}+\text{H}; ^{35}\text{Cl}, ^{37}\text{Cl}$] $^+$. HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{19}\text{ClN}_5$ [$\text{M}+\text{H}$] $^+$ 364.1324, found 364.1327.

2.3 *In vitro* *P. falciparum* growth inhibition assay

P. falciparum growth inhibition assays were carried out using an isotopic [^3H]-hypoxanthine incorporation assay, as previously described(47) . Briefly, synchronous ring-stage *P. falciparum* 3D7 (sensitive to chloroquine and other antimalarial drugs)(48) or Dd2 (resistant to chloroquine, quinine, pyrimethamine and sulfadoxine)(49) infected erythrocytes at 0.5% parasitemia and 2.5% hematocrit were seeded into 96 well tissue culture plates (Corning, USA), in triplicate wells, with different concentrations of test compounds. Vehicle only (0.5% DMSO) and the antimalarial compound chloroquine (Sigma) were used as negative and positive controls. After incubation for 48 h under standard *P. falciparum* culture conditions, [^3H]-hypoxanthine was added (0.5 μCi per well) and plates incubated for a further 24 h. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and [^3H] incorporation was determined using a 1450 MicroBeta Trilux scintillation counter (Perkin Elmer). Percentage inhibition of growth compared to DMSO controls was determined and IC_{50} values calculated using linear interpolation of inhibition curves(50). The mean IC_{50} ($\pm$ SD) was determined for three independent experiments.

2.4 Mammalian cell toxicity assays.

Neonatal foreskin fibroblast (NFF) cells were cultured in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS (CSL Biosciences, Parkville, Victoria, Australia), 1% streptomycin (Life Technologies, Inc., Rockville, MD; complete medium) at 37 °C and 5% CO_2 . Cytotoxicity assays were carried out as previously described(47) . Percentage inhibition of growth was compared to matched DMSO controls. IC_{50} values were calculated using linear interpolation of inhibition curves. The mean IC_{50} ($\pm$ SD) is shown for two independent experiments, each carried out in triplicate.

3 Results and Discussion

3.1 Hybrid compound design.

The hybrid compounds (**4**, **5**, **7**, **8**, **10** and **11**) of this study comprise the 7-chloro-4-aminoquinoline scaffold of chloroquine and a primary benzene sulfonamide scaffold of CA inhibitors, Figure 2. Hybrid compounds require a linker group to act as a spacer between the hybrid components. We selected the 1,2,3-triazole moiety as the linker group as this group is synthetically suited to covalently combining two scaffolds and is also stable to environments encountered by small molecules when added to cells and tissues such as acidic, basic, reductive and oxidative conditions as well as to enzymatic degradation.⁽⁵¹⁾ The 1,2,3-triazole is synthesized from hybrid components that comprise the complementary arrangement of azide and terminal alkyne reacting groups, using copper catalyzed azide-alkyne cycloaddition (CuAAC), also referred to as click chemistry. The 1,2,3-triazole is a nonclassical bioisostere for a peptide bond and may either participate passively (linker only) or actively (linker plus interactions with biological target) in the resulting hybrid compound.⁽⁵¹⁾ Three different linker spacings with one, two or three methylene groups directly attached to the 1,2,3-triazole, were investigated. The sulfonamide group is either *meta*- or *para*- to the 1,2,3-triazole group while in the control compounds (**6**, **9** and **12**) the sulfonamide is replaced by a hydrogen. The SAR with control compounds allows an assessment of the effectiveness of the hybrid approach with chloroquine and primary sulfonamides.

Figure 2. Structural components of 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds (**4**, **5**, **7**, **8**, **10** and **11**) and control compounds (**6**, **9** and **12**) with a 1,2,3-triazole linker.

3.2 Hybrid compound synthesis.

Compounds were synthesized in two steps from commercially available 4,7-dichloroquinoline as outlined in Scheme 1. 4,7-Dichloroquinoline was treated with an excess of propargylamine, 1-amino-3-butyne or 3-azido-propyl-1-amine and the reaction heated in a sealed tube at 110 °C until complete (17-63 h). The CuAAC reaction of **1-3** with either 4-azidobenzenesulfonamide, 3-azidobenzenesulfonamide, phenylazide, 4-ethynylbenzenesulfonamide, 3-ethynylbenzenesulfonamide or phenyl acetylene under standard conditions of 0.2 equiv. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.4 equiv. of sodium ascorbate, *t*BuOH:water 1:1, 40 °C gave triazoles **4-12**. Low reaction yields for CuAAC reactions is attributed to an extensive purification protocol implemented to remove copper ions from the aminoquinoline-triazole products.

Scheme 1. Synthetic route to 7-chloro-4-aminoquinoline hybrid compounds.

Reagents and conditions. (i) propargylamine (4.0 equiv.), 110 °C, 17 h, 72%; (ii) 1-amino-3-butyne (2.0 equiv.), Et₃N (2.0 equiv.), 110 °C, 63 h, 40%; (iii) 3-azidopropyl-1-amine.HCl (2.5 equiv.), Et₃N (5.0 equiv.); 110 °C, 24 h, 79%; (iv) azide (1.0 equiv.), alkyne (1.0 equiv.), CuSO₄·5H₂O (0.2 equiv.), sodium ascorbate (0.4 equiv.), *t*BuOH:water 1:1, 40 °C, 15-67 h, 8-63%.

3.3 *In vitro* antimalarial activity of hybrid compounds

The *in vitro* growth inhibitory activity of 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds, and control analogues lacking a primary sulfonamide moiety, was tested against a drug-sensitive (3D7) and drug-resistant (Dd2) *P. falciparum* line. Line 3D7 is sensitive to chloroquine (IC₅₀ 0.02 μM) while line Dd2 is considered resistant to chloroquine (IC₅₀ 0.08 μM). The 3D7 and Dd2 IC₅₀s for chloroquine are consistent with previous reports(52, 53). Of the compounds tested, **6** (IC₅₀ ~1 μM), **9** (IC₅₀ ~0.2-0.3 μM) and **12** (IC₅₀ ~0.6 μM) displayed the most potent inhibitory activity, however all were at least 10-fold less active than chloroquine alone (Table 1). Notably, these compounds are the control analogues that do not contain a primary sulfonamide, suggesting that the sulfonamide moiety may reduce antimalarial potency when combined with the 7-chloro-4-aminoquinoline scaffold in hybrid compounds. Interestingly the resistance index (Ri), which is the ratio of the IC₅₀s of the

resistant line Dd2 to the sensitive line 3D7, for compounds **6**, **9** and **12** was ≤ 1.4 . This Ri value is substantially lower than that obtained for chloroquine (Ri = 4.3), indicating that the addition of the phenyl-1,2,3-triazole to the aminoquinoline scaffold overcomes the change in sensitivity seen for these two lines when exposed only to chloroquine.

Of the hybrid compounds with a primary sulfonamide group either *meta*- or *para*- to the 1,2,3-triazole group, the position of the primary sulfonamide substituent had an effect on antimalarial potency. The *meta*- regioisomers (**5**, **8** and **11**) were more potent than the corresponding *para*- regioisomers (**4**, **7** and **10**) against both sensitive and resistant parasite lines. This finding suggests that the sulfonamide group has the potential to be optimized further for antimalarial potency using this hybrid compound approach. The *meta*- sulfonamide **11** was the most potent sulfonamide, with similar activity against the drug sensitive and resistant *P. falciparum* lines (IC₅₀ ~2 μ M; Ri = 0.8). In contrast, compound **10**, the *para*- regioisomer of **11**, had poorer activity for the Dd2 line resulting in a higher Ri (Ri = 3.7) than **10**, interestingly this Ri value is similar to that for chloroquine (Ri = 4.3). The structure-activity relationship between the *meta*- and *para*- sulfonamide regioisomers and the resistance index is reversed for regioisomers **4** and **5**. In this compound pair the change in position of the primary sulfonamide group from *para*- (**4**) to *meta*- (**5**) results in Ri values of 1.2 and 3.2, respectively. Finally, for regioisomers **7** and **8**, the Ri is high (Ri = 4.8 and 5.1, respectively) irrespective of the position of the primary sulfonamide substituent. For the three different linker spacings, with one (hybrids **4** and **5**), two (hybrids **7** and **8**), or three (hybrids **10** and **11**) methylene groups directly attached to the 1,2,3-triazole, the SAR was flat.

In order to examine the selectivity of 7-chloro-4-aminoquinoline:primary sulfonamide hybrids and controls for *P. falciparum* parasites versus normal mammalian cells, *in vitro* cytotoxicity assays were carried out with neonatal foreskin fibroblast (NFF) cells and the results compared to that obtained for chloroquine. In agreement with previous reports by

ourselves and others, chloroquine has a high selectivity index (SI) for parasites versus this normal mammalian cell line (SI = 301-1220)(47, 54, 55). Of the 7-chloro-4-aminoquinoline:primary sulfonamide hybrid and control analogues, control compound **9**, had the highest SI (>833) and was also the most potent in terms of its activity *P. falciparum* (IC₅₀ 0.2-0.3 μ M; Table 1). The other control compounds, **6** and **12**, also had good selectivity (SI >227 and 104, respectively) for parasites versus NFF cells (Table 1). All primary sulfonamide hybrids were relatively non-toxic to NFF cells with no inhibition observed at the highest concentration tested (250 μ M).

3.4 Conclusion

In summary we have demonstrated the utility of CuAAC for the reliable synthesis of hybrid compounds that combine two antimalarial pharmacophores. Although the panel of 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds **4**, **5**, **7**, **8**, **10** and **11** all had reduced antimalarial activity when compared to chloroquine, they did however demonstrate the potential to overcome the resistance profile of chloroquine. This SAR suggests that the hybrid compounds may act differently than chloroquine against the lines tested. An additional finding was that the *meta*- sulfonamides (**5**, **8** and **11**) were more potent than their matched *para*-sulfonamides (**4**, **7** and **10**, respectively) against both sensitive and resistant parasite lines. Given that these hybrid compounds are regiosomers they likely share the same physicochemical properties, indicating that the primary sulfonamide group may have potential to be further optimized for antimalarial activity through structural modifications of the hybrid compounds. The most potent compound examined (**9**) also had the best selectivity (SI >833) for the parasite versus a normal mammalian cell line. The potency and selectivity of compound **9**, combined with the apparent loss of cross resistance with chloroquine (R_i = 1.4 and 4.3, respectively), provide support for further investigation of **9**, including examining the activity of this compound against additional chloroquine-sensitive and resistant lines, mode of action

studies and assessment of the *in vivo* efficacy of this compound in preclinical mouse models of malaria.

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Conflicts of Interest. The authors have no conflict of interest to declare.

Figure Legends and Tables.

Figure 1. The antimalarial drug chloroquine and representative hybrid antimalarial compounds.

Figure 2. Structural components of 7-chloro-4-aminoquinoline:primary sulfonamide hybrid compounds (**4**, **5**, **7**, **8**, **10** and **11**) and control compounds (**6**, **9** and **12**) with a 1,2,3-triazole linker.

Table 1. In vitro antimalarial activity and selectivity, and cLog P values of 7-chloro-4-aminoaminoquinoline:primary sulfonamide hybrid compounds and control compounds **1-12** and chloroquine as a reference compound.

Compd	<i>P. falciparum</i> IC ₅₀ (μM)		Ri ^a	NFF IC ₅₀ (μM)	SI ^b	cLog P ^c
	3D7	Dd2				
chloroquine	0.02 (±0.001)	0.08 (±0.004)	4.3	24.1 (±4.8)	301-1220	+3.73
4	15.2 (±2.9)	18.0 (±0.3)	1.2	>250	>14	+2.68
5	4.0 (±0.3)	13.2 (±2.0)	3.2	>250	>19	+2.68
6	1.0 (±0.2)	1.1 (±0.1)	1.1	>250	>227	+3.93
7	3.9 (±0.8)	18.7 (±1.4)	4.8	>250	>13	+2.79
8	1.4 (±0.5)	7.0 (±1.2)	5.1	>250	>36	+2.79
9	0.2 (±0.04)	0.3 (±0.03)	1.4	>250	>833	+4.04
10	4.0 (±0.5)	14.8 (±0.2)	3.7	>250	>17	+2.85
11	2.0 (±0.1)	1.7 (±0.2)	0.8	>250	>125	+2.85
12	0.6 (±0.1)	0.6 (±0.02)	0.9	62.4 (±1.2)	104	+4.11

^aResistance Index (Ri) - IC₅₀ chloroquine resistant line (Dd2)/IC₅₀ chloroquine sensitive line (3D7).

The higher the Ri the higher the level of resistance. ^bSelectivity index (SI) - mammalian cell (NFF)

IC₅₀/*P. falciparum* IC₅₀. Range shown for 3D7 and Dd2 where IC₅₀ achieved or minimum SI for

compounds where NFF IC₅₀ > 250 μM. Higher SI indicated greater parasite-specific selectivity; ^c

Calculated Log P (cLog P) values using CS ChemDraw Ultra.

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Supporting Information. ^1H and ^{13}C NMR spectra of compounds **7-8, 10-11** is provided in Supporting Information.