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Synthesis, characterization and antimalarial activity of quinoline–pyrimidine hybrids

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ABSTRACT

The aim of this study was to synthesize a series of quinoline–pyrimidine hybrids and to evaluate their in vitro antimalarial activity as well as cytotoxicity. The hybrids were brought about in a two-step nucleophilic substitution process involving quinoline and pyrimidine moieties. They were screened alongside chloroquine (CQ), pyrimethamine (PM) and fixed combinations thereof against the D10 and Dd2 strains of *Plasmodium falciparum*. The cytotoxicity was determined against the mammalian Chinese Hamster Ovarian cell line. The compounds were all active against both strains. However, hybrid (**21**) featuring piperazine linker stood as the most active of all. It was found as potent as CQ and PM against the D10 strain, and possessed a moderately superior potency over CQ against the Dd2 strain (IC_{50}: 0.157 vs 0.417 μM, ~threefold), and also displayed activity comparable to that of the equimolar fixed combination of CQ and PM against both strains.

1. Introduction

Malaria claimed the lives of 0.564 million children 5 years younger in 2010.[1] The treatment of this widespread disease has become a growing therapeutic challenge due to the rapid appearance of multidrug resistant *Plasmodium falciparum* parasites.[2] *Plasmodium falciparum* species are known for causing the most severe cases and death in humans. Chloroquine and the combination pyrimethamine/sulfadoxine used to be the first line drugs in malaria treatment and prophylaxis, respectively, but are now virtually useless against *P. f.* parasites as result of this multidrug resistance.[3] A variety of cell biological explanations have been invoked for the resistance to CQ, the representative of the 4-aminoquinoline class of antimalarial drugs. Chloroquine's efflux from the digestive vacuole of resistant parasites is believed to occur by facilitated diffusion of its protonated form down to its electrochemical gradient,[4,5] and *Plasmodium falciparum* chloroquine resistant transporter's (PfCRT) mutations have been identified as the chief determinant.[6,7] These mutations result in an increased efflux from the acidic digestive vacuole (DV) to the cytosol of the parasite either as a voltage gated channel[8–10] or as a simple carrier.[11–13]

Quinoline-based antimalarial drugs such as CQ are structurally derived from quinine, a compound extracted from the bark of the cinchona tree,[14] and the proposed mechanism for their action is the formation of a toxic complex between the quinoline and ferriprotoporphyrin IX (FP), a waste product of haemoglobin digestion, inside the parasitic food vacuole.[15] Antifolates, on the other hand, are drugs exhibiting their antimalarial activity by disrupting the parasitic folic acid pathway. Of these, PM has been the most widely used. However, point mutations in the parasitic dhfr gene have wiped out its effectiveness.[16]

Quinoline ring[17,18] and aminoalkyl side chain[19,20] structural modifications are various strategies extensively explored to restore the antimalarial efficacy of the quinoline-based drugs, and thus, overcoming the *P. f.* resistance against them.

A recent but now common strategy in the search for new antimalarial drugs is the design of hybrids. A hybrid molecule is a single entity obtained by covalently linking two distinct chemical pharmacophores with multiple effects. These molecules have been introduced in anticipation that they may overcome the drug resistance problems.[21–23] New agents with improved antimalarial activity have successfully been synthesized[24–26] based on this concept, and some have already entered the clinical trial phases.[27–29]

Quinoline–pyrimidine hybrids linked through a rigid aromatic ring[30] and through flexible linear-chained diaminoalkanes[31] have been synthesized, and their antimalarial activities reported. The first cited hybrid-type displayed activity in the micromolar range, while the second possessed improved in vitro and in vivo activity in the nanomolar range. However, neither study provided data for comparison with fixed combinations of the CQ and PM to ascertain the existence of advantages of these hybrids over the

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combinations. It is noteworthy indicating that in these quinoline–pyrimidine hybrids, the pyrimidine ring was either aryl or methyl substituted.

With these considerations in mind, we synthesized 4-aminoquinoline-pyrimidine containing flexible linkers, in which the pyrimidine moiety was diaminosubstituted in 2 and 6 positions.

We herein report the synthesis, the aqueous solubility (S_w), distribution coefficient (log$D$) and antimalarial activity of these hybrids in comparison with those of CQ, PM and of various combinations of two drugs, in an attempt to ascertain the advantages of the hybrids, if any, over these combinations.

2. Materials and methods

2.1. Materials

4,7-Dichloroquinoline and 2,6-diamino-4-chloropyrimidine were purchased from Hangzhou Dayangchem Co., Ltd (China). 2-Aminoethan-1-ol, 2-aminopropan-1-ol, 3-aminopropan-1-ol, 2-(2-aminoethoxy)ethan-1-ol, 4-aminobutan-1-ol, 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, piperazine and 1,4-diaminobenzene were purchased from Sigma–Aldrich, Ltd. HPLC grade acetonitrile was obtained from Labchem South Africa. All the reagents and chemicals were of analytical grade.

2.2. General procedures

Thin-layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck) and silica gel 60 (70–230 mesh ASTM, Fluka).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer (at a frequency of 600.17 and 150.913 MHz, respectively) in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane (TMS) as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets) and m (multiplet).

The high resolution electron spray ionisation mass spectra (HRMS) were recorded on a Waters Synapt G2 spectrometer. The source was electrospray positive, capillary voltage 3 kV, cone voltage 15 V. Introduction with direct injection (1 μl) into a stream of 50% acetonitrile, 0.1% formic acid, using a Waters UPLC at flow rate of 0.2 ml/min. Positive ions $[M+H]^+$ were recorded.

Thermo Scientific Nicolet iS10 spectrometer with Avatar diffuse reflectance accessory, Merck KBr as blank, and Omnic version 8.2 (Thermo Fischer Scientific) Software, were used for infrared (IR) analysis. The peak identification was done at sensitivity 50.

The melting points (mp) were determined on a Stuart Melting Point SMP 10 and given in degrees Celsius (°C) uncorrected.

2.3. High performance liquid chromatography (HPLC)

The HPLC system consisted of an Agilent 1100 series auto sampler, Agilent 1100 series variable wave detector (VWD) and Agilent 1100 series isocratic pump. A Zorbax Eclipse XDB C18, 5 μm (150 × 4.60 mm) column was used and the Agilent Chemstation Rev. A08.03 for LC systems software package for data analysis.

The compounds were quantified using a gradient method (A = 0.2% triethylamine in H_2O, pH 7.0, B = acetonitrile) at a flow rate of 1 ml/min with 20 μl standard sample injections. The gradient consisted of 25% of solvent B (ACN) until 1 min, then increased linearly to 95% of B after 10 min, and held for 15 min, where after the instrument was re-equilibrated to the starting conditions for 5 min. A calibration plot of peak area versus drug concentration for each compound showed excellent linearity ($0.993 < r^2 \leqslant 1$) over the concentration range (0–2000 μg/ml) employed for the assays. The absorption maximum for compounds (**12**)–(**21**) as well as for pyrimethamine was at 210 nm; this wavelength was consequently used for the HPLC detection. New mobile phase was prepared for each sample batch that was analysed by HPLC. The retention time (t_R) is expressed in minutes (min).

2.4. Synthesis of quinoline–pyrimidine hybrids

The synthesis of quinoline–pyrimidine hybrids followed a two-step process (scheme 1). In the first step, either hydroxy- or amino-functionalized quinoline intermediates (**2**)–(**6**) and (**7**)–(**11**), respectively, were synthesized (See Supplementary data).[32,33] In the second step, a given intermediate was treated with 2,6-diamino-4-chloropyrimidine in the presence of sodium hydrid to afford the target hybrids and the process is described as follows:

A mixture of intermediate (**2**)–(**11**) (1 mmol) and sodium hydride (NaH, 10 mmol, 10 equiv) was stirred in DMF at room temperature for 1 h then heated at 135 °C, and 2,6-diamino-4-chloro-pyrimidine (5 mmol; 5 equiv) was added portion wise over 30 min, and the reaction was continued for another 16–20 h. The progress of the reaction was monitored by thin layer chromatography. After completion, the mixture was span to dryness, the residue was dissolved in MeOH and purified by flash chromatography on silica gel eluting with MeOH/DCM (4:1, v/v) to afford the target compounds. The physical and spectroscopic data of compounds (**12**)–(**21**) are reported.

2.5. 6-{2-[(7-Chloroquinolin-4-yl)amino]ethoxy}pyrimidine-2,4-diamine (12)

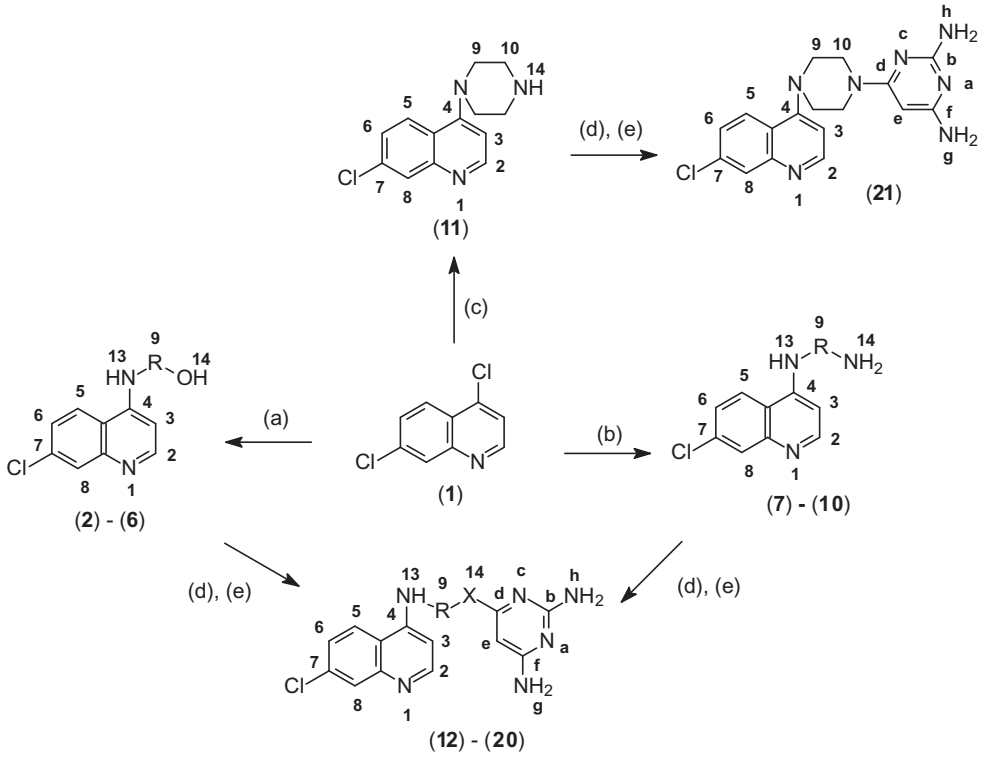
Yield: 51%; off-white powder; mp: 203–205 °C; IR (KBr)/cm^{-1}: 3506, 3340, 3141, 2964, 2782, 1984, 1739, 1653; ^{1}H NMR (600 MHz, DMSO) δ 9.22 (d, J = 5.4 Hz, 1H, H-2), 9.05 (d, J = 9.0 Hz, 1H, H-5), 8.60 (d, J = 2.0 Hz, 1H, H-8), 8.33 (s, 1H, H-13), 8.24 (dd, J = 2.0, 9.0 Hz, 1H, H-6), 7.39 (d, J = 5.4 Hz, 1H, H-3), 6.86 (s, 2H, H-g), 6.72 (s, 2H, H-h), 5.87 (s, 1H, H-e), 5.17 (t, J = 5.6 Hz, 2H, H-10), 4.39 (t, J = 5.6 Hz, 2H, H-9); ^{13}C NMR (151 MHz, DMSO) δ 179.50 (C-d), 175.63 (C-f), 172.50 (C-b), 161.61 (C-2), 159.72 (C-4),143.11 (C-7), 137.02 (C-8), 133.83 (C-6), 133.67 (C-5), 108.46 (C-3), 85.95 (C-e), 71.95 (C-10), 51.61 (C-9); $t_{R\ (HPLC)}$: 3.64 min; HRMS m/z: 331.1076 (M+1) [(M+1), $C_{15}H_{16}ON_6Cl$, 331.1074 Calcd], 333.1051 [(M+1) + 2].

2.5.1. 6-{2-[(7-Chloroquinolin-4-yl)amino]propoxy}pyrimidine-2,4-diamine (13)

Yield: 46%; off-white powder; mp: 220–221 °C; ^{1}H NMR (600 MHz, DMSO) δ 8.43 (d, J = 5.4 Hz, 1H, H-2), 8.21 (d, J = 9.0 Hz, 1H, H-5), 7.77 (d, J = 1.9 Hz, 1H, H-8), 7.55 (dd, J = 1.9, 9.0 Hz, 1H, H-6), 7.40 (s, 1H, H-13), 6.64 (d, J = 5.4 Hz, 1H, H-3), 6.02 (s, 2H, H-g), 5.90 (s, 2H, H-h), 5.31–5.25 (m, 1H, H-9), 5.01 (s, 1H, H-e), 3.49 (dt, J = 6.2, 13.0 Hz, 1H, H-10a), 3.37 (d, J = 13.0 Hz, 1H, H-10b), 1.28 (d, J = 7.2 Hz, 3H, H-11); ^{13}C NMR (151 MHz, DMSO) δ 169.60 (C-d), 166.05 (C-f), 162.88 (C-b), 152.06 (C-2), 150.03 (C-4), 133.37 (C-7), 127.50 (C-8), 124.09 (C-6), 124.01 (C-5), 99.01 (C-11), 76.86 (C-e), 67.90 (C-10), 47.52 (C-9), 18.31 (C-11); $t_{R\ (HPLC)}$: 3.67 min; HRMS m/z: 345.1230 (M+1) [(M+1), $C_{16}H_{18}ON_6Cl$, 345.1231 Calcd], 347.1202 [(M+1) + 2].

2.5.2. 6-{3-[(7-Chloroquinolin-4-yl)amino]propoxy}pyrimidine-2,4-diamine (14)

Yield: 46%; brown powder; mp: 228–230 °C; IR (KBr)/cm^{-1}: 3484, 3317, 3163, 2972, 2947, 2895, 2207, 1740, 1631; ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, J = 5.4 Hz, 1H, H-2), 8.25 (d, J = 9.0 Hz, 1H, H-5), 7.78 (d, J = 2.4 Hz, 1H, H-8), 7.44 (dd, J = 2.4, 9.0 Hz, 1H, H-6), 7.34 (s, 1H, H-13), 6.47 (d, J = 5.4 Hz, 1H, H-3),



Comp.	R	X	Comp.	R	X	Comp.	R	X
(**12**)	$^{9\ 10}CH_2CH_2$	O	(**15**)	$^{9\ 10\ 11\ 12}CH_2CH_2OCH_2CH_2$	O	(**18**)	$^{9\ 10\ 11}CH_2CH_2CH_2$	NH
(**13**)	$^{9\ 11\ 10}CH(CH_3)CH_2$	O	(**16**)	$^{9\ 11\ 12\ 10}CH(CH_2CH_3)CH_2$	O	(**19**)	$^{9\ 10\ 11\ 12}CH_2CH_2CH_2CH_2$	NH
(**14**)	$^{9\ 10\ 11}CH_2CH_2CH_2$	O	(**17**)	$^{9\ 10}CH_2CH_2$	NH	(**20**)		NH

Scheme 1. A general reaction illustrating the synthesis of quinoline–pyrimidine hybrids (**12**)–(**20**) and (**21**). Reagents and conditions: (a) 4,7-dichloroquinoline (**1**), aminoalcohol, neat, 120 °C, 24 h; (b) 4,7-dichloroquinoline (**1**), diaminealkane/aryldiamine, neat or DMF, 80–150 °C, 24 h; (c) 4,7-dichloroquinoline (**1**), piperazine, DMF, 80–135 °C, 5 h; (d) functionalized quinoline (**2**)–(**10**) and (**11**), NaH, DMF, room temperature, 1 h, then (e) 2,6-diamino-4-chloropyrimidine, DMF, 135 °C, 16–24 h.

6.03 (s, 2H, H-g), 5.85 (s, 2H, H-h), 5.07 (s, 1H, H-e), 4.19 (t, *J* = 6.7 Hz, 2H, H-11), 3.36 (t, *J* = 6.1 Hz, 2H, H-9), 2.01 (dt, *J* = 6.1, 6.7 Hz, 2H, H-10); ^{13}C NMR (151 MHz, DMSO) δ 170.12 (C-d), 166.06 (C-f), 163.02 (C-b), 151.97 (C-2), 150.16 (C-4), 133.49 (C-7), 127.48 (C-8), 124.17 (C-6), 124.13 (C-5), 98.73 (C-3), 76.20 (C-e), 62.62 (C-11), 39.44 (C-9), 27.62 (C-10); t_R (HPLC): 3.73 min; HRMS *m*/*z*: 345.1229 (M+1) [(M+1), $C_{16}H_{18}ON_6Cl$, 345.1231 Calcd], 347.1205 [(M+1) + 2].

2.5.3. 6-2-{2-[(7-Chloroquinolin-4-yl)amino]ethoxy}ethoxy) pyrimidine-2,4-diamine (15)

Yield: 53%; off-white powder; mp: 109–110 °C; IR (KBr)/cm^{-1}: 3479, 3396, 3365, 3296, 3173, 2570, 2405, 1911, 1640; ^{1}H NMR (600 MHz, DMSO) δ 8.38 (d, *J* = 5.3 Hz, 1H, H-2), 8.24 (d, *J* = 9.0 Hz, 1H, H-5), 7.78 (s, 1H, H-8), 7.44 (d, *J* = 9.0 Hz, 1H, H-6), 7.33 (s, 1H, H-13), 6.51 (d, *J* = 5.3 Hz, 1H, H-3), 6.01 (s, 2H, H-g), 5.87 (s, 2H, H-h), 5.03 (s, 1H, H-e), 4.22 (t, *J* = 7.5 Hz, 2H, H-12), 3.69 (t, *J* = 7.5 Hz, 4H, H-10, -11), 3.46 (t, *J* = 7.5 Hz, 2H, H-9); ^{13}C NMR (151 MHz, DMSO) δ 169.85 (C-d), 166.02 (C-f), 162.90 (C-b), 151.95 (C-2), 150.05 (C-4), 133.41 (C-7), 127.53 (C-8), 124.14 (C-6), 124.03 (C-5), 98.79 (C-3), 76.19 (C-e), 68.95 (C-12), 68.12 (C-11), 63.85 (C-10), 42.29 (C-9); t_R (HPLC): 3.41 min; HRMS *m*/*z*: 375.1330 (M+1) [(M+1), $C_{17}H_{20}O_2N_6Cl$, 375.1336 Calcd], 377.1310 [(M+1) + 2].

2.5.4. 6-{2-[(7-Chloroquinolin-4-yl)amino]butoxy}pyrimidine-2,4-diamine (16)

Yield: 45%; cream-white powder; mp: 221–223 °C; ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, *J* = 5.5 Hz, 1H, H-2), 8.33 (d, *J* = 9.0 Hz, 1H, H-5), 7.77 (d, *J* = 2.2 Hz, 1H, H-8), 7.42 (dd, *J* = 2.2, 9.0 Hz, 1H, H-6), 7.02 (s, 1H, H-13), 6.60 (d, *J* = 5.5 Hz, 1H, H-3), 6.02 (s, 2H, H-g), 5.89 (s, 2H, H-h), 5.00 (s, 1H, H-e), 4.31 (dd, *J* = 6.8, 10.9 Hz, 1H, H-10a), 4.13 (dd, *J* = 5.3, 10.9 Hz, 1H, H-10b), 3.89–3.78 (t, *J* = 5.9 Hz, 1H, H-9), 1.73–1.60 (m, 2H, H-11), 0.92 (t, *J* = 7.4 Hz, 3H, H-12); ^{13}C NMR (151 MHz, DMSO) δ 170.10 (C-d), 166.14 (C-f), 163.02 (C-b), 152.11 (C-2), 150.38 (C-4), 133.70 (C-7), 127.48 (C-86), 124.41 (C-64), 124.21 (C-5), 99.27 (C-3), 76.46 (C-e), 66.12 (C-10), 53.29 (C-9), 23.94 (C-11), 10.55 (C-12); t_R (HPLC): 3.99 min; HRMS *m*/*z:* 359.1388 (M+1) [(M+1), $C_{17}H_{20}ON_6Cl$, 359.1387 Calcd], 361.1357 [(M+1) + 2].

2.5.5. 4-*N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}pyrimidine-2,4,6-triamine (17)

Yield:45%; light yellow crystals; mp: 229–230 °C; IR (KBr)/cm^{-1}: 3435, 3330, 3138, 2941, 2206, 1930, 1894; ^{1}H NMR (600 MHz,

DMSO) δ 8.40 (d, J = 5.4 Hz, 1H, H-2), 8.18 (d, J = 9.0 Hz, 1H, H-5), 7.77 (d, J = 2.2 Hz, 1H, H-8), 7.59 (s, 1H, H-13), 7.39 (dd, J = 2.2, 9.0 Hz, 1H, H-6), 6.52 (d, J = 5.4 Hz, 1H, H-3), 6.45 (s, 1H, H-14), 5.61, (s, 2H, H-g), 5.55 (s, 1H, H-h), 4.87 (s, 1H, H-e), 3.46 (t, 2H, J = 5.6 Hz, H-10), 3.33 (t, J = 5.6 Hz, 2H, H-9); ^{13}C NMR (151 MHz, DMSO) δ 164.26 (C-d), 163.99 (C-f), 162.92 (C-b), 152.09 (C-2), 150.10 (C-4), 133.36 (C-7), 127.43 (C-8), 124.14 (C-5), 124.05 (C-6), 98.61 (C-3), 74.36 (C-e), 43.77 (C-10), 38.47 (C-9); t_R (HPLC): 3.50 min; HRMS m/z: 330.1231 (M+1) [(M+1), $C_{15}H_{17}N_7Cl$, 330.1234 Calcd], 332.1206 [(M+1) + 2].

2.5.6. 4-*N*-{3-[(7-Chloroquinolin-4-yl)amino]propyl}pyrimidine-2,4,6-triamine (18)

Yield: 36%; light yellow powder; mp: 196–197 °C; IR (KBr)/cm^{-1}: 3489, 3389, 3148, 2918, 2880, 2565, 1764; ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, J = 5.4 Hz, 1H, H-2), 8.25 (d, J = 9.1 Hz, 1H, H-5), 7.77 (d, J = 2.2 Hz, 1H, H-8), 7.43 (dd, J = 2.2, 9.1 Hz, 1H, H-6), 7.30 (s, 1H, H-13), 6.46 (dd, J = 5.4 Hz, 1H, H-3), 6.14 (s, 1H, H-14), 5.54 (s, 2H, H-g), 5.35 (s, 2H, H-h), 4.84 (s, 1H, H-e), 3.29 (t, J = 6.2 Hz, 2H, H-11), 3.18 (t, J = 6.6 Hz, 2H, H-9), 1.85–1.75 (m, 2H, H-10); ^{13}C NMR (151 MHz, DMSO) δ 164.34 (C-d), 163.97 (C-f), 162.92 (C-b), 151.96 (C-20), 150.09 (C-4), 133.39 (C-7), 127.49 (C-8), 124.10 (C-5), 124.06 (C-6), 98.72 (C-3), 73.97 (C-e), 40.31 (C-11), 38.22 (C-9), 27.84 (C-10); t_R (HPLC): 3.59 min; HRMS m/z: 344.1386 (M+1) [(M+1), $C_{16}H_{19}N_7Cl$, 344.1390 Calcd], 346.1363 [(M+1) + 2].

2.5.7. 4-*N*-{4-[(7-Chloroquinolin-4-yl)amino]butyl}pyrimidine-2,4,6-triamine (19)

Yield: 25%; off-white powder; mp: 159–160 °C; ^{1}H NMR (600 MHz, DMSO) δ 8.37 (d, J = 5.3 Hz, 1H, H-2), 8.25 (d, J = 9.1 Hz, 1H, H-5), 7.77 (d, J = 1.5 Hz, 1H, H-8), 7.43 (dd, J = 1.5, 9.1 Hz, 1H, H-6), 7.30 (s, 1H, H-13), 6.46 (t, J = 5.3 Hz, 1H, H-3), 6.05 (s, 1H, H-14), 5.54 (s, 2H, H-g), 5.34 (s, 2H, H-h), 4.83 (s, 1H, H-e), 3.27 (t, J = 6.4 Hz, 2H, H-12), 3.10 (t, J = 7.3 Hz, 2H, H-9), 1.70–1.63 (m, 2H, H-10), 1.62–1.55 (m, 2H, H-11). ^{13}C NMR (151 MHz, DMSO) δ 164.31 (C-d), 163.95 (C-f), 162.90 (C-b), 151.97 (C-2), 150.09 (C-4), 133.37 (C-7), 127.49 (C-8), 124.12 (C-5), 123.99 (C-6), 98.70 (C-11), 73.90 (C-e), 42.24 (C-12), 40.04 (C-9), 26.89 (C-10), 25.41 (C-11); t_R (HPLC): 3.72 min; HRMS m/z: 358.1541 (M+1) [(M+1), $C_{17}H_{21}N_7Cl$, 358.1546 Calcd], 360.1514 [(M+1) + 2].

2.5.8. 4-*N*-{4-[(7-Chloroquinolin-4-yl)amino]phenyl}pyrimidine-2,4,6-triamine (20)

Yield: 13%; dark yellow powder; mp: 222–223 °C; IR (KBr)/cm^{-1}: 3439, 3324, 3191, 2977, 2182, 1623; ^{1}H NMR (600 MHz, DMSO) δ 8.98 (s, 1H, H-13), 8.60 (s, 1H, H-14), 8.42 (d, J = 5.4 Hz, 1H, H-2), 8.38 (d, J = 8.9 Hz, 1H, H-5), 7.85 (d, J = 2.2 Hz, 1H, H-8), 7.66 (d, J = 8.0 Hz, 2H, H-10), 7.52 (dd, J = 2.2, 8.9 Hz, 1H, H-6), 7.34 (J = 8.0 Hz, 2H, H-11), 6.68 (d, J = 5.4 Hz, 1H, H-3), 5.81 (s, 2H, H-g), 5.64 (s, 2H, H-h), 5.21 (s, 1H, H-e); ^{13}C NMR (151 MHz, DMSO) δ 164.66 (C-d), 162.93 (C-f), 161.47 (C-b), 151.92 (C-2), 149.55 (C-4), 138.88 (C-20), 133.78 (C-23), 132.26 (C-15), 127.60 (C-16), 124.63 (C-13), 124.46 (C-22 & C-24), 124.37 (C-14), 120.01 (C-21 & C-25), 117.91 (C-18), 100.80 (C-11), 76.37 (C-e); t_R (HPLC): 3.97 min; HRMS m/z: 378.1233 (M+1) [(M+1), $C_{19}H_{17}N_7Cl$, 378.1234 Calcd], 380.1195 [(M+1) + 2].

2.5.9. 6-[4-(7-Chloroquinolin-4-yl)piperazine-1-yl]pyrimidine-2,4-diamine (21)

Yield: 60%; yellow crystals; mp: 154–155 °C; IR (KBr)/cm^{-1}: 3484, 3312, 3155, 2941, 2275, 1921; ^{1}H NMR (600 MHz, DMSO) δ 8.71 (d, J = 5.1 Hz, 1H, H-2), 8.10 (d, J = 9.0 Hz, 1H, H-5), 7.99 (d, J = 2.2 Hz, 1H, H-8), 7.56 (dd, J = 2.2, 9.0 Hz, 1H, H-6), 7.03 (d, J = 5.1 Hz, 1H, H-3), 5.77 (s, 2H, H-g), 5.54 (s, 2H, H-f), 5.12 (s, 1H, H-e), 3.25 (t, J = 6.3 Hz, 4H, H-10), 3.20 (t, J = 6.3 Hz, 4H, H-9); ^{13}C NMR (151 MHz, DMSO) δ 165.32 (C-d), 163.76 (C-f), 162.76 (C-b), 156.28 (C-2), 152.23 (C-4), 133.62 (C-7), 128.06 (C-8), 126.14 (C-5), 125.86 (C-6), 109.57 (C-3), 74.21 (C-e), 51.56 (C-10), 43.64 (C-9); t_R (HPLC): 3.75 min; HRMS m/z: 356.1389 (M+1) [(M+1), $C_{17}H_{19}N_7Cl$, 356.1312 Calcd], 358.1369 [(M+1) + 2].

2.6. Physicochemical properties

2.6.1. Solubility

The aqueous solubility (S_w) of crystalline compounds (**12**)–(**21**) as well as PM was obtained by preparing solutions in PBS (pH 5.5 and 7.4). The slurries were stirred in a water bath at 37 °C for 24 h. An excess of solute was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analyzed directly by HPLC to determine the concentration of solute dissolved in the solvent.[34] The experiment was performed in triplicate. The S_w values are listed in Table 1.

2.6.2. Experimental log*D*

Equal volumes of *n*-octanol and PBS (pH 5.5) were mixed with vigorous stirring for at least 24 h. Two milligram of each derivative was dissolved in 0.75 mL of this solution, the solution was then stoppered and agitated for 10 min in 2 mL graduated tubes (0.5 mL division). Subsequently, 0.75 mL of pre-saturated buffer was transferred to the tubes containing the mentioned solutions. The tubes were stoppered and agitated for 45 min after which they were centrifuged at 4000 rpm (1503 g) for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min, where after their volume ratio (v/v; *n*-octanol/buffer) was determined. The volume ratio was found in all cases to be one. The *n*-octanol and aqueous phases were then analyzed by HPLC. From this data, the concentrations of the derivative in both phases were determined. The log*D* values were calculated as logarithmic ratios of the concentrations in the *n*-octanol phase compared to the concentrations in the buffer.[34] The experiment was performed in triplicate. Experimental log*D* values were also determined using PBS pH 7.4. All the results expressed as means are listed in Table 1.

2.7. In vitro biological studies

2.7.1. Antimalarial activity

The samples were tested in triplicate on one occasion against chloroquine-susceptible (CQS) D10 and chloroquine-resistant (CQR) Dd2 strains of *Plasmodium falciparum*. Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[35] The quantitative assessment of in vitro antimalarial activity was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler and co-workers.[36]

The test samples were prepared as a 2 mg/mL stock solution in 10% dimethyl sulfoxide (DMSO) and sonicated to enhance solubility. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine was used as the reference drug in all experiments. A full-dose response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50}). The same dilution technique was used for all samples. The samples were tested in triplicate. The solvents to which the parasites were exposed had no measurable effect on the parasite viability. The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software, and the values on molar basis in Table 2 were obtained by dividing those on mass basis by the molecular weight of each compound.

Table 1
Aqueous solubility, distribution coefficients and lipid solubility of hybrids (**12**)–(**21**) and PM for each compound, aqueous solubility (S_w) was determined in PBS (pH 5.5 and 7.4) at 37 °C after incubation in a water bath with stirring for 24 h; distribution coefficient ($\log D$) was obtained after *n*-octanol/PBS (pH 5.5 and 7.4) partition

Compd	$S_{w\ 5.5}$[a] (μM)	SD	$\log D_{5.5}$[a]	SD	$S_{OC\ 5.5}$[b] (μM)	$S_{w\ 7.4}$[a] (μM)	SD	$\log D_{7.4}$[a]	SD	$S_{OC\ 7.4}$[b] (μM)
(**12**)	0.30	0.00	−0.30	0.04	0.15	0.23	0.01	0.90	0.04	1.84
(**13**)	1.45	0.09	−1.34	0.19	0.07	0.25	0.02	0.92	0.03	2.14
(**14**)	0.31	0.02	−0.08	0.01	0.25	0.12	0.01	1.01	0.05	1.2
(**15**)	1.17	0.07	−0.29	0.03	0.60	0.84	0.01	−0.28	0.04	0.44
(**16**)	0.04	0.01	1.07	0.10	0.49	0.02	0.00	2.47	0.05	5.68
(**17**)	0.52	0.03	−0.07	0.01	0.45	0.12	0.01	0.78	0.07	0.72
(**18**)	0.88	0.08	−0.74	0.02	0.16	0.62	0.01	0.11	0.04	0.79
(**19**)	1.04	0.01	−0.74	0.08	0.19	0.94	0.06	0.49	0.01	2.91
(**20**)	0.01	0.00	nd[c]			nd[c]		nd[c]		
(**21**)	0.66	0.02	−0.99	0.07	0.07	0.01	0.00	nd[c]		
PM	1.16	0.03	0.76	0.02	6.67	0.15	0.00	1.93	0.20	12.83

[a] Experimental, data represent the mean of three independent measurements.
[b] Solubility in octanol (S_{OC}) calculated from experimental S_w and $\log D$ using $\log S_{OC} = \log D + \log S_w$.
[c] Compound not water soluble enough to determine value, not determined (nd).

Table 2
In vitro antimalarial activity of quinoline–pyrimidine hybrids (**12**)–(**21**), pyrimethamine, chloroquine and combinations **M1** and **M2** against D10 and Dd2 strains of *Plasmodium falciparum*

Compd	D10						Dd2					RI[f]
	n[a]	Mean IC_{50}[b] (M)	Std. Dev.	*p*-Value Welch	*p*-Value Dunnett[c]		*n*[a]	Mean IC_{50}[b] (M)	Std. Dev.	*p*-Value Welch	*p*-Value Dunnett[c]	
					CQ	PM					CQ	
(**12**)	3	0.230	0.026		0.002[d]	0.009[d]	3	0.580	0.248		0.729	2.6
(**13**)	3	0.243	0.015		0.001[d]	0.004[d]	3	0.543	0.117		0.911	2.3
(**14**)	3	0.217	0.006		0.003[d]	0.018[d]	3	0.467	0.025		1.000	2.2
(**15**)	3	0.837	0.129		0.000[d]	0.000[d]	3	4.103	0.266		0.000[d]	5.0
(**16**)	3	0.157	0.006		0.088	0.322	3	0.210	0.046		0.472	1.4
(**17**)	3	0.317	0.015		0.000[d]	0.000[d]	3	1.021	0.140		0.000[d]	3.2
(**18**)	3	0.850	0.132		0.000[d]	0.000[d]	3	1.207	0.189		0.000[d]	1.4
(**19**)	3	0.580	0.010		0.000[d]	0.000[d]	3	1.263	0.232		0.000[d]	2.2
(**20**)	3	0.220	0.000		0.003[d]	0.015[d]	3	0.107	0.006		0.101	0.5
(**21**)	3	0.070	0.017		0.996	1.000	3	0.157	0.006		0.229	2.4
PM	3	0.070	0.010	0.000			3	[e]				
CQ	3	0.040	0.010	0.000			3	0.417	0.067	0.000		10.4
M1	3	7.77 ng/ml	0.41				3	47.5 ng/ml	5.7			6.1
M2	3	8.43 ng/ml	0.45				3	165.33 ng/ml	17.79			19.6

Cells were incubated with compounds at various concentrations for 48 h and the antimalarial activity was determined using parasite lactate dehydrogenase assay.
[a] Number of replicates.
[b] Data represents the mean of three independent experiments.
[c] Statistical significance at 0.05 level.
[d] *p*-Value <0.05 indicates a statistically significant difference between IC_{50} values with more than 95% certainty.
[e] Value above maximum tested concentration.
[f] Resistance index (RI) = IC_{50}Dd2/IC_{50}D10.

2.7.2. Cytotoxicity

The MTT-assay is used as a colorimetric assay for cellular growth and survival and compares well with other available assays.[37,38] The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The test samples were tested in triplicate on one occasion. The same stock solution which was used for the antiplasmodial assay was used for the cytotoxicity assay. Stock solutions were stored at −20 °C until use. Dilutions were prepared on the day of the experiment. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/ml, which was serially diluted in complete medium with 10-fold dilutions to give six concentrations, the lowest being 0.001 μg/ml. The same dilution technique was applied to all the test samples. The highest concentration of solvent to which the cells were exposed had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4 software. The results are listed in Table 3.

2.8. Statistical analysis of antiplasmodial activity and cytotoxicity

One-way analyses of variances (ANOVA) were performed for data on the D10 and Dd2 strains to determine if significant differences existed between the mean IC_{50} values of hybrids and combinations and those of CQ and PM in general. Levene's tests were performed to assure equality of variances in each ANOVA's case. In the case of inequality of variances, Welch tests were performed. Normal probability plots on the residuals were done to assure that the data was fairly normally distributed.[39] Dunnett's tests were done to determine which of the test compounds' mean differ statistically significantly from the mean of the standard drugs CQ and PM. Tukey's post hoc test, on the other hand, was done to determine which of the test compounds' mean differ statistically significantly from each other, omitting the reference drugs from the analysis. These procedures were done using the statistical data analysis software system.[40] All tests were done at a 0.05 significant level. The statistical methods utilised to determine the cytotoxicity

Table 3
Cytotoxicity results of hybrids (12) -(21), pyrimethamine, combinations M1, M2 and emetine against CHO cells. Cells were incubated with compounds at various concentrations for 48 h and the cytotoxicity was determined using MTT-assay.

Compd	n[a]	CHO				SI[f]
		Mean IC_{50}[b] (M)	Std. Dev.	p-Value Welch	p-Value: Dunnett[d] EM	
(**12**)	3	173.93	21.345		0.021[e]	764.38
(**13**)	3	250.00	0.000		0.005[e]	1030.05
(**14**)	3	107.213	10.336		1.000	494.33
(**15**)	3	250.0	0.000		0.005[e]	303.36
(**16**)	3	155.873	3.711		0.950	995.21
(**17**)	3	189.717	72.858		0.309	607.28
(**18**)	3	254.98	21.505		0.004[e]	308.09
(**19**)	3	162.38	13.69		0.862	279.86
(**20**)	3	13.85	3.738		0.015[e]	62.96
(**21**)	3	146.043	80.642		0.997	2073.54
PM	3	[c]				
M1	3	67.6 g/ml	17.5			8700
M2	3	89.7 g/ml	14.04			10,650
EM[†]		124.83	20.805	0.000[e]		

[a] Number of replicates.
[b] Data represents the mean of three independent experiments.
[c] Value above maximum tested concentration.
[d] Statistical significance at 0.05 level.
[e] p-Value <0.05 indicates a statistically significant difference between IC_{50} values with more than 95% certainty.
[f] Selectivity index (RI) = IC_{50}CHO/IC_{50}D10.

of the test compounds against CHO were similar to those used for antiplasmodial activity testing using emetine (EM) as reference drug.

3. Results

3.1. Chemistry

The IR spectra of (**2**)–(**6**) showed characteristic absorption in the region 3200–3100 cm^{-1} attributed to the hydroxy group. This functional group was confirmed in the structures by the presence of a singlet in the 5–4.8 ppm region in the ^1H NMR spectra due to OH proton. The IR spectra of intermediates (**7**)–(**11**) commonly showed the presence of absorption bands in the 3360–3250 cm^{-1} region, due to the NH_2 and NH groups. The presence of the primary amino NH_2 group was also confirmed in the structures of (**7**)–(**10**), and (**11**) by the singlet due to signal of the exchangeable NH_2 and NH protons, respectively, ca. ~3 ppm in the ^1H spectra.

The hybrids (**12**)–(**16**) and (**17**)–(**21**), oxo and amino linked, respectively, were afforded in yields varying from low (13%) to moderate (60%). Their chemical structures were confirmed by IR, NMR and MS analysis. The IR spectra of hybrids (**12**)–(**15**), (**17**), (**18**), (**20**) and (**21**) exhibited broad vibrational bands in the 3450–3300 cm^{-1} region due to the two NH_2 substituents on the pyrimidine ring. The band ca. ~3100 cm^{-1} is attributed to the NH group in the linkers.

In the ^1H NMR spectra, the quinoline moiety was clearly identified by the presence of four doublets and one doublet of doublet assigned to signals of the heterocyclic protons H-2, H-3, H-5, H-8 and H-6, respectively. The peaks at 152.09, 150.10, 133.36, 127.43, 124.14, 124.05 and 98.61 ppm corresponded to signal of carbon C-2, C-4, C-7, C-8, C-5, C-6 and C-3, respectively, in the ^{13}C spectra, which further confirmed this fact.

The pyrimidine ring was also present in the structures of the hybrids as evidenced by a singlet in the 5.5–4.0 ppm region, which corresponds to the signal of the solitary heterocyclic proton H-e. The protons of NH_2 substituents in positions 2 (b) and 6 (f) were also confirmed by the presence of two distinctive singlets of exchangeable protons H-h and H-g in the 7–5.5 ppm region. The peaks in the ^{13}C spectra at ca. ~165, ~163 and ~162 ppm assigned to the signals of carbon C-d, C-f and C-b, respectively, and the distinctive signal ~75 ppm of carbon C-e further corroborate the presence of that moiety.

Furthermore, the mass spectra of all the hybrids showed two molecular ion peaks $[M+H]^+$ and $[(M+H)+2]^+$. These data supported the identity of the compounds and confirmed the presence of one chlorine atom in their structures. It is important to mention that under our work-up, no dimeric compounds were isolated.

3.2. Aqueous solubility (S_w) and experimental log$D$

Aqueous solubility and lipophilicity influence the way a molecule passes through biological membranes and barriers to ultimately enter the systemic circulation. Drugs must thus possess balanced lipophilic/hydrophilic properties to both permeate biological membranes and be taken up for systemic circulation. The logarithm of ratio of octanol solubility to water solubility (log$P$) is a good measure of this balance with values between 0 and 5 being targeted, and 0–3 being ideal[41,42]

The experimentally determined distribution coefficients (log$D$), pH dependant version of the partition coefficient, are the logarithmic ratios of octanol solubility to buffer at a given pH value, and serve as an indication of an investigated compound's behavior in vivo. The octanol mimics the biological membrane while the PBS buffer represents the physiological environment and the cytosol/digestive vacuole of the parasite, at pH 7.4 and 5.5, respectively. The lipid solubility indicated by the solubility in octanol was deduced using the equation: $\log S_{OC} = \log D + \log S_w$ in which log$D$ and S_w are the experimental data. All the results are summarized in Table 1.

The data revealed each hybrid to exhibit slightly higher aqueous solubility at pH 5.5 than at pH 7.4, and inversely, a higher lipophilicity at pH 7.4 than at 5.5. Hybrids (**13**), (**15**) and (**19**) were found to be as hydrophilic as PM at pH 5.5 while (**14**) and (**15**) had water solubilities similar to that of PM in the neutral environment. None of the hybrids was as lipophilic as PM irrespective of the medium considered.

3.3. Biological evaluation

3.3.1. In vitro antimalarial activity

The quinoline–pyrimidine hybrids as well as the combinations **M1** (CQ/PM, 1:1) and **M2** (CQ/PM, 1:4) were screened in vitro alongside CQ and PM against the CQ-susceptible D10 and -resistant Dd2 strains, two clones of the human *Plasmodium falciparum* malaria, and were all found active.

Comparing the antimalarial activity of the hybrids to that of CQ, Dunnett's test revealed the mean IC_{50} values of compounds (**12**)–(**15**) and (**17**)–(**20**) to differ statistically significantly from that of CQ since they have p-values smaller than 0.05, implying that these hybrids were less potent than CQ against the D10 strain (Table 2). On the contrary, the hybrids (**16**) and (**21**) were found to be as potent as CQ against the same strain. Their mean IC_{50} values were not statistically significantly different from that of CQ.

Moreover, hybrids (**15**) and (**17**)–(**20**) were less potent than CQ while (**12**)–(**14**), (**16**), (**20**) and (**21**) as well as the combinations proved to be as potent against Dd2 as CQ according to the aforementioned test.

In comparison to PM, the hybrids (**12**)–(**15**) and (**17**)–(**20**) were less potent while the hybrids (**16**) and (**21**) showed comparable potency against D10 strain. PM was inactive against the Dd2 strain, thus all the hybrids proved to be more potent than PM, which happened to be inactive against that strain.

Hybrid (**15**) was unequivocally the least active of all. Hybrid (**18**) on the other hand was also less active than all other hybrids

and combinations against D10, but was found to be as potent as (**17**) and (**19**) against Dd2. Hybrid (**19**) had a mean IC_{50} value statistically significantly different and was found to be more active than (**15**) and (**18**) but displayed less antiplasmodial activity than all other synthesized compounds and the combinations against the D10 strain (Table 4, Supplementary statistical data).

However, it was more active than (**15**), as active as both (**17**) and (**18**), and was found less active than the rest of hybrids against the resistant strain Dd2 (Table 5, Supplementary statistical data). The pairs of hybrids **(12)** and (**21**), and (**16**) and (**17**), on the other hand, have Tukey *p*-value of 0.053, which indicates a 94.7% certainty that their mean IC_{50} values are statistically significantly different. This implies that compound (**12**) was less active than (**21**) while (**16**) was more active than (**17**) against the D10 strain. Similarly, the mean IC_{50} values of compounds (**13**) and (**20**) are statistically significantly different a 95% certainty (*p*-value = 0.050). Thus, hybrid (**13**) was less active than (**20**) against the Dd2 strain.

Overall, (**20**) and (**21**) stood as the most active hybrids and displayed comparable activities against the CQR strain. However, the comparison of the RI values (0.49 vs 2.36) indicates (**20**) carried its activity against D10 over to Dd2, whereas (**21**) lost a fivefold activity instead.

Comparison of the activity reveals no difference between the combinations against D10. **M1** was thus found to be as active as **M2**; against Dd2, however, the equimolar combination **M1** was more active than **M2**. Although both combinations lost activity against the resistant strain, this fact was more pronounced with **M2** than **M1** as seen from the difference in RI values (6.1 vs 19.6).

3.3.2. Cytotoxicity

The hybrids and combinations were very selective in their antimalarial action against the parasitic cells in the presence of the host CHO cells as can be seen from the SI high values (Supplementary Table 5). Thus, they were all non-toxic to the mammalian cells. The Dunnett's test clearly reveals hybrids (**14**), (**16**), (**17**), (**19**) and (**21**) as cytotoxic as emetine (*p*-values >0.05), while the cytotoxicity of (**12**), (**13**), (**15**), (**18**) and (**20**) were statistically significantly different from that of emetine (*p*-values <0.05). Hybrid (**20**) had the lowest SI value of all compounds including emetine, and thus appears to be less selective to the parasitic cells than the reference. The data (Table 6, Supplementary statistical data) confirm that the cytotoxicity of that hybrid was not only the lowest but also statistically significantly different from that of all other compounds.

In summary, hybrid (**21**) stood as the best of all synthesized compounds with respect to both the antimalarial activity and the selectivity towards the parasitic cells.

Comparing the cytotoxicity, the SI values revealed hybrid (**21**) to be less selective to parasitic cells than both combinations; an indication that the mammalian cells are safer in the presence of the combinations than the hybrid (**21**).

4. Discussion

4.1. Chemistry

The hybrids were obtained in a two-step process. Both steps involved nucleophilic substitutions (S_NAr type) and the same nucleofuge (chlorine ion). The first step was not catalyzed whereas the second step giving rise to the hybrids was through the use of sodium hydrid. As a rule, the best leaving groups in nucleophilic substitution reactions are weak bases, and chloride ion is one of them.[43] Quinoline and pyrimidine are two basic aromatic rings. However, the basicity of the pyrimidine is reduced by the presence of the two amino substituents in 2 and 6 positions. This ultimately leads to the chloride atom being a stronger base than the one on the quinoline, and therefore more strongly retained on the pyrimidine ring causing it to be less reactive towards weak nucleophiles.

Furthermore, a negatively charged nucleophile is always a more reactive specie than its conjugated acid.[43] Subsequently, negatively charged nucleophiles generated from the quinoline intermediates had to be used in order to displace the chlorine of 2,6-diamino-4-chloropyrimidine to afford the target hybrids. Thus, sodium hydrid (NaH) was employed to induce in situ alkoxide RO^- and aminides RNH^-/RN^- from R-OH and R-NH_2/NH, respectively, during the first hour of the second step.

The intermediates possess the ionizable groups NH linked to quinoline ring, (except in **11**) and terminal OH in (**2**)–(**6**), NH_2 in (**7**)–(**10**), and NH in (**11**). The rates and yields of S_N2 reactions are influenced, among others by the concentration of the nucleophile.[43] In an attempt, to insure high yields, NaH was used in fivefold excess, which resulted in the formation of alkoxide and/or aminide byproducts, and may explain the low to moderate yields obtained. The highest yield, hybrid (**21**) from (**11**), which the only monoprotic intermediate with the potential to deliver a single aminide (no byproduct formed), supports this explanation.

4.2. Physicochemical properties

Chloroquine and pyrimethamine are basic molecules that are protonated at pH levels lower than the physiological pH of 7.4 (CQ: pK_a 8.38 and 10.18,[44] and PM pK_a 7.36[45]). This is in accordance with the ability of the quinolines and PM to permeate biological membranes at physiological pH (unionized form) and accumulate in high concentrations in the acidic environment of the malaria parasite's food vacuole (ionized form).[46] Thus, the synthesized hybrids should possess, high hydrophilicity in the acidic condition of pH 5.5 (protonated) and high lipophilicity at the physiological pH 7.4 (unprotonated). The results of this study support these facts. Indeed, although not very significant, the differences between S_w values showed each hybrid to display better hydrophilicity in the acidic than in the neutral medium. Hybrid (**20**), featuring a phenyl linker, was the least hydrophilic of all, and this is presumably due to its inability to protonate in either medium apart from the linker being notoriously lipophilic. Compounds (**13**) and (**19**) were the most hydrophilic at pH 5.5 and 7.4, respectively. On the contrary, compound (**15**) was the most lipophilic in the acidic medium while hybrid (**16**) displayed the highest lipophilicity in the neutral conditions. Compound (**15**) features two ethylene oxide units in the linker, which may explain its good solubility in both media as a result of H-bonding between the water molecules and the intra chain oxygen atoms of ethylene oxide.

Moreover, for a given linker, the amino linked hybrids tend to be slightly more water soluble than their oxo linked counterpart in the acidic medium, presumably as a result of additional protonation apart from the hydrogen bonding. Indeed, the amine-linked hybrids possess a diprotic nature in the acidic medium. While both hybrid-types can be protonated at the quinoline ring nitrogen (pK_a 8.1) at pH 5.5, the amine hybrids undergo an additional protonation at their terminal nitrogen.[47] Thus, hybrid (**17**) was more water soluble than (**12**) when considering the ethyl linker. The same applied for (**18**) and (**14**) for the propyl linker. However, this tendency can only be firmly confirmed upon investigation of much broader series of compounds

4.3. Antimalarial activity and cytotoxicity

The synthesized hybrids were tested in vitro and were not metabolised by metabolic enzymes, thus acting as new entities and not as prodrugs. The observed IC_{50} values are therefore more likely to be those of the hybrids and not of any active metabolite.

Hybrid (**21**), the most active, featuring piperazine linker was as potent as CQ against both the D10 and Dd2 strains, and was the most selective towards parasitic cells. However, this hybrid did not display better solubility and distribution coefficient values compared to CQ, which is reported to possess S_w and log D values of 0.03 mM and 4.3, respectively, in acidic (pH 5.5) medium.[48,49] The activity of (**21**) may be due to the compound accumulating in higher concentration in the digestive vacuole as result of the piperazine-linker being protonated more strongly despite these less favourable properties. The activity of (**21**), thus appeared unrelated to its solubility. This is in accordance with the early finding that the drug's absorption is dependent upon multiple physicochemical parameters.[50]

All the hybrids were more potent than PM against the Dd2, which proved to be inactive against that strain. Thus, the activity of these compounds was linked solely to the quinoline pharmacophore. With the exception of (**20**) and (**21**), which displayed moderate potency (two- and threefold, respectively) over CQ against Dd2, no other hybrid possessed better antimalarial activity than CQ. This suggests that the pyrimidine pharmacophore antagonizes the quinoline in these less performing hybrids.

Furthermore, hybrids (**20**) and (**21**) contained the rigid aromatic and piperazine linkers, respectively, and yet, are the most active of all, which indicates the activity of these compounds to be unrelated to the flexibility of the linker between the two pharmacophores. This is also in accordance with the literature.[31]

In summary, hybrid (**21**) showed antimalarial efficacy in the micromolar range and better selectivity against both strains, and also indicates an improved activity of the two molecules (quinoline and pyrimidine) in the hybrid form as compared to the individual form.

The combination **M1** was as potent as **M2** against the D10 strain but more than **M2** against Dd2. Both combinations showed comparable selectivity towards the parasitic cells, which indicates that **M2** had no tangible advantages over **M1**.

Another objective of this study was to ascertain the advantages of the hybrids, if any, over the combinations. The activity clearly demonstrated that there were none. Indeed, (**21**) the most active hybrid was found with IC_{50} = 0.07 and 0.157 μM, which translate into activity of 25 and 56 ng/ml (vs 7.77 and 47.5 ng/ml for **M1**) against D10 and Dd2, respectively. These data indicate (**21**) to be statistically as potent as the combination **M1** against both strains while being less selective towards the parasitic cells. Thus, hybrid (**21**) did not display any advantages over the equimolar combination of CQ and PM.

Comparing the activity of (**21**) with the sum of the activity of the individual drugs reveals no difference against the D10 strain. Thus, the actions of CQ and PM in this hybrid were additive. Against the Dd2, however, there was a significant difference observed. The hybrid displayed activity slightly superior to the combined activity of CQ and PM when applied individually indicating a weak synergism against the Dd2 strain.

This study was not intended to structure activity relationships as the number of hybrids, ten in total (5 oxo- and 5 amine-linked) was too small to draw realistic conclusions based on such an analysis.

5. Conclusion

A series of quinoline–pyrimidine hybrids was synthesized, and their structures were validated by means of NMR and MS analyses. The aqueous solubility and distribution coefficient determined experimentally revealed the hybrids to be more water soluble at pH 5.5 than 7.4, and inversely displayed higher lipophilicity at pH 7.4 than 5.5. The hybrids were all active against both D10 and Dd2 strains, and showed good selectivity towards these parasitic cells. Hybrid (**21**), which features a piperazine linker, was the most active of all. This compound was as potent as both chloroquine and pyrimethamine against the sensitive strain, possessed superior potency over both drugs against the resistant strain, and was found with potency similar to that of the equimolar combination thereof against Dd2. The actions of these two drugs were additive and weakly synergistic through the hybrid (**21**) against the D10 and Dd2, strains, respectively. Thus, this hybrid appears worthy of being further investigated to ascertain whether the in vitro micromolar activity could be carried over in vivo, and if it operates either as quinoline or folate or both, in our search for new potent antimalarials.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.019.

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