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Targeting Asexual and Sexual Blood Stages of the Human Malaria Parasite *P. falciparum* with 7-Chloroquinoline-Based 1,2,3-Triazoles

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Novel 4-amino-7-chloroquinoline-based 1,2,3-triazole hybrids were synthesised in good yields by Cu^{I}-catalysed Huisgen 1,3-dipolar cycloaddition reactions of 2-azido-*N*-(7-chloroquinolin-4-ylaminoalkyl)acetamides with various terminal alkynes. These new hybrids were screened in vitro against asexual blood stages of the chloroquine-sensitive 3D7 strain of *P. falciparum*. The most active compounds were further screened against asexual and sexual stages (gametocytes) of the chloroquine-resistant RKL-9 strain of *P. falciparum*. Although all compounds were less potent than chloroquine against the 3D7 strain, the three best compounds were appreciably more active than chloroquine against the RKL-9 strain, displaying IC_{50} values of <100 nM, with one of them having an IC_{50} of 2.94 nM. Further, the lead compounds were gametocytocidal with IC_{50} values in the micromolar range, and were observed to induce morphological deformations in mature gametocytes. Most compounds demonstrated little or no cytotoxicity and exhibited good selectivity indices. The most active compounds represent promising candidates for further evaluation of their schizonticidal and gametocytocidal potential.

Introduction

Malaria is a tropical parasitological disease that remains one of the biggest global health challenges despite the availability of effective control and treatment measures. Severe malaria caused by *P. falciparum* is the major cause of mortality and morbidity worldwide, especially in sub-Saharan Africa.[1] Antimalarial drugs have long been a mainstay in the fight against this deadly disease, whether as chemoprophylactic agents or as part of the treatment regimen. However, efforts to eliminate malaria have been jeopardised by the continuous emergence of resistance to common antimalarial drugs including chloroquine (CQ), sulfadoxine–pyrimethamine and recently artemisinin.[2] This problem is further compounded by the lack of an effective transmission-blocking drug with a good safety profile for glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals.[3] Therefore, development of safe and effective antimalarial drugs including those with transmission blocking potential is crucial for tackling the ongoing threat of drug resistance and to curb the transmission of malaria.[4] In order to widen the scope of treatment and support the malaria elimination program of the World Health Organization, developing antimalarial drugs with broader therapeutic potentials that can simultaneously target both asexual blood stages that cause disease symptoms and gametocytes, responsible for transmission, is beneficial. Historically, the most targeted pathway for antimalarial drug development is detoxification of haem and formation of haemozoin.[5] A number of highly effective 4-aminoquinoline antimalarial drugs such as CQ and amodiaquine function by targeting this pathway. Structure–activity studies of 4-aminoquinolines have suggested that the presence of a chloro group at the 7-position and a terminal amino group is required for antimalarial activity, and their potency is enhanced by the presence of a basic side chain attached to the amino group, such as in CQ.[6–8] Further modification of this basic side chain has led to improved antimalarials with high potency against *P. falciparum*.[9] Moreover, studies on 4-aminoquinoline analogues also resulted in the generation of promising lead compounds,[10–12] and several candidates are in preclinical development or clinical trials.[13] Considering the safety and efficacy of 4-aminoquinoline-based drugs and the remarkable success of CQ in the past, the 4-aminoquinoline moiety is pharmaceutically suitable for use as a scaffold to develop new antimalarial drug candidates.

However, compounds based solely on this 4-aminoquinoline moiety might exhibit similar cross-resistance susceptibility patterns as shared by other established 4-aminoquinolines such as CQ and amodiaquine, and might illicit drug resistance responses earlier in geographical areas reporting CQ resistance. Therefore, in order to broaden the structural diversity of the compounds, with the additional objective of intensifying biological activity, covalently linked hybrids were created with an-

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other pharmacologically significant class of compounds—1,2,3-triazoles—appended with a 7-chloro-4-aminoquinoline base. These 1,2,3-triazoles, exhibit myriad biological activities including antifungal,[14] antibacterial[15] and antitubercular activities.[16] Apart from being a passive linker, the triazoles can also act as important constituent of antimalarial compounds[17] because they are lipophilic nitrogen-containing heterocycles that tend to accumulate into the food vacuole of the parasite, a property also exhibited by CQ.[18] Prototypes of reported quinoline-based triazole hybrids synthesised by other investigators, along with their antimalarial activity (IC_{50}), are shown in Figure 1. 1,2,3-Triazole-tethered 7-chloroquinoline and β-lactam bifunctional hybrids in which a 1,2,3-triazole links the β-lactam ring (connected at the 4-position of the triazole

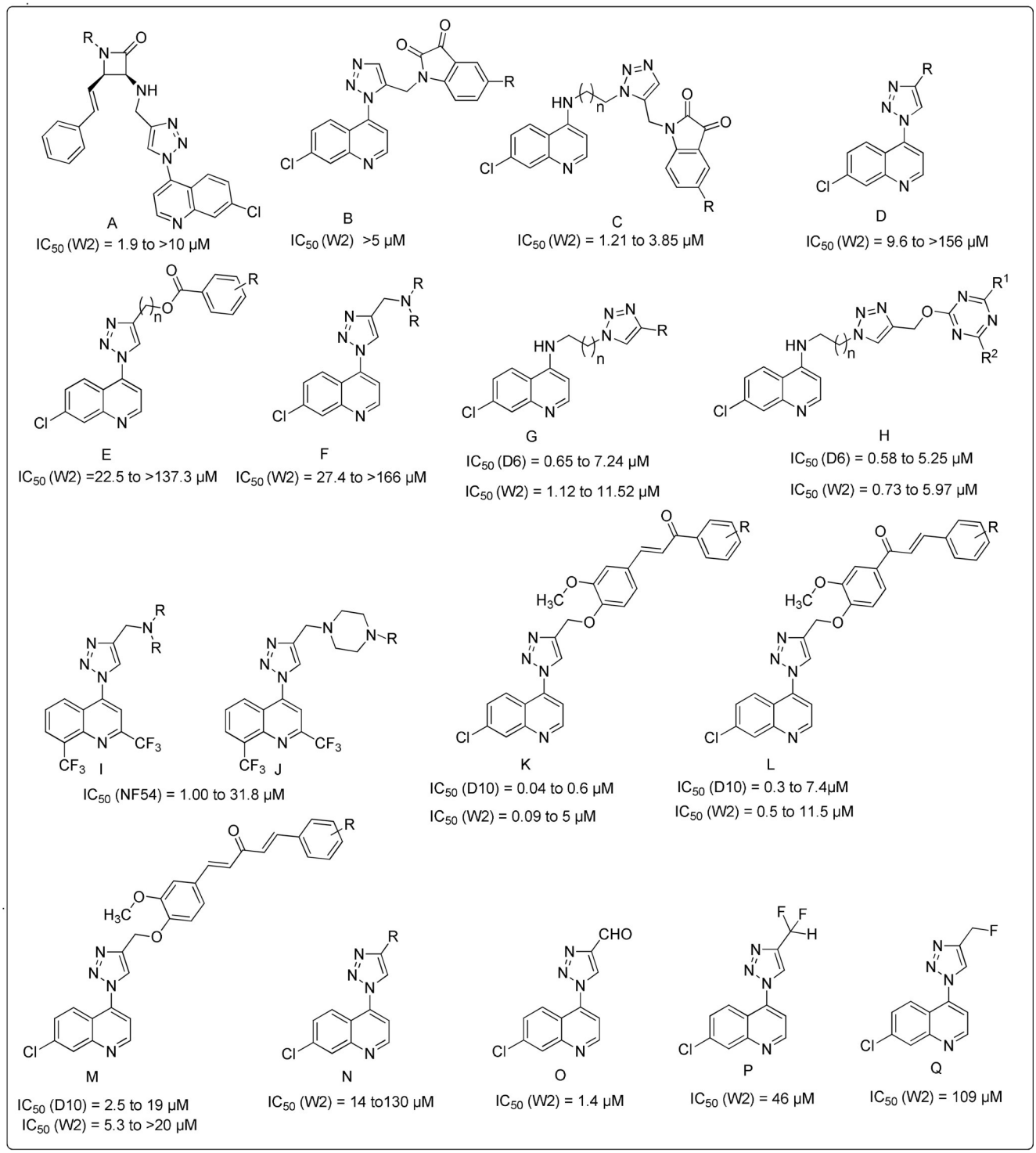
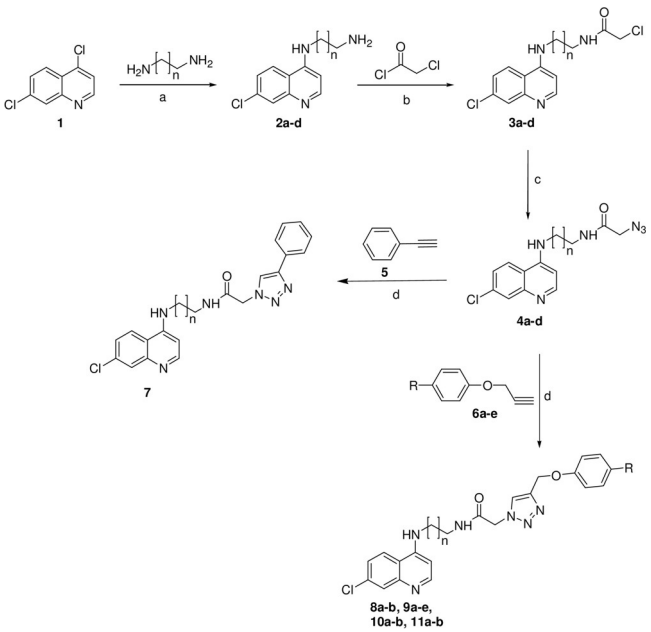


Figure 1. Structures and IC_{50} values of quinoline- and triazole-based hybrids reported by Singh et al.[19] (A), Raj et al.[20] (B and C), Pereira et al.[21] (D–F), Rawat et al.[22] (G and H), Hamann et al.[23] (I and J), Guantai et al.[24] (K–M) and Boechat et al.[25] (N–Q). The identities of the R groups and other details can be found in the respective references.

through an aminomethyl group) with the 7-chloroquinoline moiety, demonstrated IC_{50} values in the range of 1.9 to >10 μM for the CQ-resistant W2 strain of *P. falciparum* (Figure 1, structure A).[19] Enhanced potency of 1,2,3-triazole-tethered 7-chloroquinoline–isatin hybrids (Figure 1, structure C) was observed if the 1,2,3-triazole is linked to the 7-chloroquinoline skeleton by an aliphatic amine linker as compared with hybrids in which the 1,2,3-triazole is covalently bonded to the 7-chloroquinoline base (Figure 1, structure B).[20] The most potent molecule, 5-chloro-1-{1-[3-(7-chloroquinolin-4-yl-amino)-propyl]-1*H*-1,2,3-triazol-4-ylmethyl}-1*H*-indole-2,3-dione, demonstrated an IC_{50} of 1.21 μM against the W2 strain of *P. falciparum*.[20] Furthermore, copper-catalysed cycloaddition reactions were carried out to yield hybrids containing a 1,2,3-triazole moiety (having different substituents) directly attached to a 7-chloroquinoline base. These compounds displayed potency in the range of 9.6 to >166 μM against the W2 strain of *P. falciparum* (Figure 1, structures D–F).[21] Rawat et al. evaluated the antiplasmodial activity of 4-amino-7-chloroquinoline-1,2,3-triazole and 4-amino-7-chloroquinoline-1,2,3-triazole–triazine hybrids (Figure 1, structures G and H) in which the triazole moiety is appended to the 7-chloro-4-aminoquinoline base by an aliphatic linker. IC_{50} values for the 4-amino-7-chloroquinoline-1,2,3-triazole and 4-amino-7-chloroquinoline-1,2,3-triazole–triazine hybrids were reported to be in ranges 0.65 to 7.24 μM and 0.58 to 5.25 μM for the CQ-sensitive D6 strain, respectively, and 1.12 to 11.52 μM and 0.73 to 5.97 μM for the CQ-resistant W2 strain, respectively.[22] The concept of a triazole as a linker between two moieties was further extended to the synthesis of compounds with mefloquine (Figure 1, structures I and J) instead of the standard 7-chloroquinoline; IC_{50} values were reported to be in range 1.00 to 31.8 μM for the NF54 strain of *P. falciparum*.[23] Guantai and co-workers developed antimalarial compounds (Figure 1, structures K to M) in which 1,2,3-triazoles acted as linkers between a chalcone functionality (connected at the 4-position of the triazole through an ether linkage) and a 7-chloroquinoline moiety, with IC_{50} values from 0.04 to 19 μM for the CQ-sensitive D10 strain and from 0.09 to >20 μM for the CQ-resistant W2 strain of *P. falciparum*.[24] Moreover, 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines (Figure 1, structures N to Q) with different substituents at the 4-position of the 1,2,3-triazole ring were synthesised, and half-maximal inhibitory concentrations were reported to be in the range 1.4 to 130 μM against the CQ-resistant W2 strain of *P. falciparum*.[25] In the majority of these studies, the 1,2,3-triazole moiety is either covalently bonded to the quinoline scaffold, without any linker[19–21,23–25] or it is appended with 7-chloroquinoline through an aliphatic aminoalkyl chain.[20,22] Inspired by the previously reported work, and consistent with our aim to develop novel antimalarials based on quinoline-triazole scaffold, we designed hybrid compounds in such a way that the 1,2,3-triazole group is linked to 7-chloroquinoline by an aliphatic diamine linker through a carbonyl methylene group (quinoline–NH–R–$NHCOCH_2$–triazole, where R is an aliphatic group; Scheme 1). The advantage of hybrid molecules is their ability to surpass the threat of drug resistance due to the presence of multiple pharmacophores and hence multiple mechanism of actions.



Scheme 1. Synthesis of 7-chloroquinoline-based 1,2,3-triazole hybrids **7**, **8a**,**b**, **9a**–**e**, **10a**,**b** and **11a**,**b**: a) 130 °C, 7–8 h, 83–91%; b) DMF, RT, 5–6 h, 78–96%; c) NaN_3, MeOH, reflux, 7–8 h, 79–91%; d) $CuSO_4$, sodium ascorbate, *t*BuOH/H_2O (1:1), RT, 8 h, 76–88%.

With the advent of new antimalarial drugs, our aim was to obtain novel chemical entities with two "biologically privileged" pharmacophores, coupled into a single molecular framework that can act at more than one target site to intensify the antimalarial activity. We herein report the synthesis, characterisation and evaluation of antimalarial activity of a panel of novel 4-amino-7-chloroquinoline-based 1,2,3-triazole hybrids.

Results and Discussion

Synthesis

A new series of 4-amino-7-chloroquinoline-based 1,2,3-triazole hybrids were synthesised by Cu^I-catalysed Huisgen [3+2] cycloaddition reaction of 2-azido-*N*-(7-chloroquinolin-4-ylamino-alkyl)acetamides **4a**–**d** and terminal alkynes **5** and **6a**–**e** in a *t*BuOH/H_2O (1:1) mixture containing a catalytic amount of copper sulfate and sodium ascorbate at ambient temperature (Scheme 1). Initially, alkynes **6a**–**e** were prepared in one step by reacting substituted phenols with propargyl bromide in the presence of K_2CO_3 in DMF at room temperature for 8–10 h.[26,27] The desired azides **4a**–**d** were synthesised in three steps. Firstly, *N*′-(7-chloroquinolin-4-yl)alkanediamines **2a**–**d** were obtained by heating alkanediamines with 4,7-dichloroquinoline (**1**) at 130 °C for 7–8 h, according to a literature procedure.[28,29] In the next step, the free amino groups of **2a**–**d** were coupled with chloroacetyl chloride in DMF at ambient temperature to afford compounds **3a**–**d**, which on heating with sodium azide in methanol at reflux for 7–8 h afforded the desired precursors **4a**–**d** in almost quantitative yields. Finally, the syntheses of the desired compounds **7**, **8a**,**b**, **9a**–**e**, **10a**,**b** and **11a**,**b** were ac-

complished in good to excellent yields by Cu^{I}-catalysed Huisgen [3+2] cycloaddition reactions between 2-azido-*N*-(7-chloroquinolin-4-ylaminoalkyl)acetamides **4a–d** and terminal alkynes **5** and **6a–e** in the presence of a catalytic amount of copper sulfate and sodium ascorbate in 50% aqueous *t*-butanol at ambient temperature (Scheme 1). The structures of all the compounds were confirmed on the basis of spectral analysis and their characterisation data are presented in the Experimental Section.

The IR spectrum of the representative compound **9e** showed four characteristic absorption peaks at 3274, 1686, 1656 and 1599 cm^{-1}, corresponding to the stretching frequencies of NH, C=O, CHO and C=N, respectively. In the ^{1}H NMR spectrum of **9e**, four characteristic peaks were observed at $\delta =$ 9.86, 8.21, 5.28 and 5.12 ppm, which correspond to the protons of CHO, triazole, CH_2O and CH_2CO groups, respectively. Mass spectral analysis provided further evidence for the formation of *N*-[3-(7-chloroquinolin-4-ylamino)propyl]-2-[4-(4-formylphenoxymethyl)-1,2,3-triazol-1-yl]acetamide (**9e**) with the presence of a $[M+H]^+$ ion peak at *m/z* 479, corresponding to a molecular formula of $C_{24}H_{23}ClN_6O_3$. The other compounds were similarly analysed and their characterisation data are presented in the Experimental Section.

Antimalarial activity against the asexual stages of a CQ-sensitive strain (3D7) of *P. falciparum* and structure–activity relationships

Initial screening of these 7-chloroquinoline-triazole hybrids for antimalarial activity was performed using a malaria SYBR Green I fluorescence (MSF) assay. All 12 compounds demonstrated antimalarial activity in the nanomolar range (38.75 to 910.14 nM) against the 3D7 strain of *P. falciparum*. Of the tested compounds, the three most potent compounds—**8a**, **8b** and **9c**—demonstrated IC_{50} values of 39.98, 40.00 and 38.75 nM, respectively (Table 1). The in vitro screening results revealed that the antimalarial activity of these hybrid molecules generally decreases with an increase in the length of the aliphatic diamino linker that connects the 1,2,3-triazole and 7-chloroquinoline scaffolds. In addition, the presence of a halogen at the *para* position of the phenoxy group within a series having same spacer is crucial for the antimalarial activity of the synthesised compounds, with the exception of compound **9c**. Overall, the molecules having lipophilic substituents such as Cl, Br and CH_3 at the *para* position of the phenoxy ring were found to be more active than those having either an unsubstituted phenoxy substituent or a *para*-substituted phenoxy ring with the more hydrophilic formyl substituent (Table 1).

Selectivity assays

The selectivity of 12 new compounds for *P. falciparum* (3D7) versus VERO cells was determined using an MTT assay, with the selectivity index (SI) defined as the ratio of CC_{50} and IC_{50} values (Table 1). In general, the majority of compounds showed no significant toxicity. Compound **11a** showed the lowest SI, whereas **8a** demonstrated the highest SI of all the compounds. On the basis of a collective analysis of potency against the CQ-sensitive strain (3D7) of *P. falciparum* and toxicity data, the three compounds showing a promising selectivity profile—**8a**, **8b** and **9c**—were also tested against asexual stages of CQ-resistant *P. falciparum* and against sexual stages, the details of which are mentioned in later sections.

Table 1. In vitro antimalarial activity and cytotoxicity of 4-amino-7-chloroquinoline-based 1,2,3-triazoles.

Compd	R	*n*	Asexual stage		Gametocyte stage		Cytotoxicity	
			3D7	RKL-9	RKL-9		VERO	
			IC_{50} [nM]	IC_{50} [nM] (95% CI)	IC_{50} [μM] (NM) (95% CI)	IC_{50} [μM] (total) (95% CI)	CC_{50} [μM][a]	SI[b]
7	–	2	137.10	–	–	–	41.53	304.78
8a	Cl	1	39.98	75.01 (60.53–92.96)	10.71 (7.70–14.88)	20.87 (17.30–25.16)	86.75	2223.72
8b	Br	1	40.00	2.94 (1.96–4.43)	8.50 (6.76–10.67)	19.80 (15.06–26.02)	28.86	723.477
9a	H	2	179.56	–	–	–	78.21	437.81
9b	Cl	2	90.02	–	–	–	40.58	453.68
9c	Br	2	38.75	16.01 (10.84–23.65)	12.03 (10.05–14.3)	21.91 (17.79–26.98)	27.06	713.62
9d	CH_3	2	91.94	–	–	–	75.49	828.12
9e	CHO	2	910.14	–	–	–	>208	>236.47
10a	Cl	3	92.77	–	–	–	12.18	132.42
10b	CH_3	3	83.55	–	–	–	10.87	130.52
11a	Cl	5	263.12	–	–	–	5.02	19.08
11b	Br	5	307.29	–	–	–	7.41	24.19
CQ	–	–	5.8	114.4 (77.70–168.4)	–	–	112.44	>20000

[a] CC_{50}: concentration of test compound required to inhibit VERO cell growth by 50%. [b] Selectivity index (CC_{50}/IC_{50}) of 3D7 strain. CI: confidence interval; NM: normal morphology.

Antimalarial activity against the asexual stages of CQ-resistant field isolate (RKL-9) and structure–activity relationships

CQ resistance has spread globally,[30] especially in *P. falciparum*, and almost all malaria-prone areas are gradually becoming CQ resistant, therefore it is imperative to evaluate if these compounds showed signs of cross-resistance with CQ, because they also possess the 7-chloroquinoline moiety. Therefore, the antimalarial activities of compounds **8a**, **8b** and **9c** were evaluated against a CQ-resistant field isolate collected from one of the most malaria-endemic areas of India (Rourkela, Odisha). Compounds **8a**, **8b** and **9c** exhibited IC_{50} values of <100 nM against CQ-resistant field isolate (RKL-9) of *P. falciparum* (Table 1). Of these three compounds, compound **8b** was found to be most potent, as evident from its IC_{50} value of 2.94 nM, the lowest of the three, followed by **9c** with an IC_{50} of 16.01 nM. Compound **8a** was found to be the least active with an IC_{50} value 75.01 nM. The respective dose–response curves are shown in Figure S1 in the Supporting Information.

These results revealed that the replacement of a halogen moiety (from chloro to bromo) at the *para* position of the phenoxy group remarkably increases the potency of the compound against CQ-resistant *P. falciparum* by approximately 25-fold (IC_{50} 2.94 vs. 75.01 nM). Moreover, an ethyl side chain (instead of propyl) attached to the 4-amino-7-chloroquinoline moiety increases the antimalarial potency by approximately fivefold (IC_{50} 2.94 vs. 16.01 nM). Although compounds **8a**, **8b** and **9c** structurally resemble CQ owing to the presence of a 7-chloroquinoline moiety, the potency data demonstrates that none exhibit cross-resistance with CQ, thereby demonstrating comparatively higher potency than CQ (the IC_{50} of which is 114.4 nM) against CQ-resistant *P. falciparum*. Collectively, compounds **8a**, **8b** and **9c** demonstrated greater potency than CQ against CQ-resistant *P. falciparum*, and the fact that they also proved effective against CQ-sensitive *P. falciparum* makes them potential candidates to be developed further as schizonticidal antimalarial agents.

Antimalarial activity against gametocytes

For carrying out the drug sensitivity experiments, mature gametocytes were produced from the same field isolate (RKL-9) that was used for measuring the asexual stage antimalarial activity. The compounds **8a**, **8b** and **9c** were clearly found to target mature gametocytes and induce morphological deformation in a dose-dependent manner. Compound **8b** showed maximum potency with an IC_{50} (NM) (normal morphology, see the Experimental Section) of 8.50 μM, and was considered the most active of the three tested compounds. Compounds **8a** and **9c** also targeted gametocytes with IC_{50} (NM) values 10.71 and 12.03 μM, respectively. IC_{50} (NM) and IC_{50} (total) values for the tested compounds are presented in Table 1. The respective dose–response curves are shown in Figure S2. Microscope images of morphological deformation induced by treatment with these hybrids, along with images of healthy/untreated gametocytes are shown in Figure S3 in the Supporting Information.

Conclusions

In summary, 4-amino-7-chloroquinoline-based 1,2,3-triazole hybrids were synthesised by using a click chemistry approach and were screened for their potential antiplasmodial activity. All the compounds demonstrated promising activity in the nanomolar range against a CQ-sensitive strain (3D7) of *P. falciparum*. Three hybrids (**8a**, **8b** and **9c**) were also found to be active against a CQ-resistant field isolate of *P. falciparum* (RKL-9) and were more potent than CQ itself, suggesting the minimum possibility of cross-resistance with CQ. Furthermore, these compounds also demonstrated potency against mature gametocytes, which suggests their potential transmission blocking activity. These compounds also exhibited no evidence of cytotoxicity against the mammalian cell line VERO. Overall, the promising schizonticidal and gametocytocidal activity displayed by the most active quinoline–triazole hybrids described here encourage further studies to identify newer hybrids, especially as most quinoline-based schizonticidal drugs are relatively inactive against mature gametocytes.[38] Collectively, these results indicate that the new hybrids containing a carbonyl group bonded to an alkylamino linker connecting a 4-amino-7-chloroquinoline base and 1,2,3-triazole might be considered prototypes for the development of next-generation antimalarial agents.

Experimental Section

Materials and methods

All chemicals were purchased from Sigma–Aldrich unless otherwise stated and were used without further purification. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ pre-coated aluminium plates from Merck, and spots were visualised either under UV light or in an iodine chamber. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in [D_6]DMSO on a Jeol ECX-400P (400 MHz) NMR spectrometer. The coupling constants *J* are reported in Hertz (Hz). IR spectra were recorded on a PerkinElmer IR spectrometer and absorption maxima ν_{max} are reported in cm^{-1}. Mass spectra were recorded on a Waters Micromass LCT ESI-MS spectrometer in positive-ion mode. Elemental analyses were conducted on an Elementar Analysensysteme GmbH VarioEL V3.00. Melting points were determined in open capillary tubes on a Büchi melting point apparatus and are uncorrected. N^1-(7-Chloroquinolin-4-yl)alkanediamines **2a–d** were prepared according to the literature methods and their characterisation data matched the reported data.[28,29]

General procedure for the synthesis of compounds 3a–d

Chloroacetyl chloride (4.0 mmol) was added to a well stirred solution of an N^1-(7-chloroquinolin-4-yl)-alkanediamine (**2a–d**; 1.0 mmol) in DMF (10 mL), and the reaction mixture was stirred at ambient temperature for 4–5 h. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (40 mL) and the pH of the solution was adjusted to 10 by addition of 10% aqueous NaOH solution. The resulting solution was then extracted with ethyl acetate (60 mL×3). The ethyl acetate layers were combined, washed with water (40 mL×3), dried over anhydrous Na_2SO_4 and then evaporated under vacuum to afford the

products (**3a–d**) in good yields and in sufficiently pure forms to be used in further reactions without purification.

2-Chloro-*N*-[2-(7-chloroquinolin-4-ylamino)ethyl]acetamide (3a): Dark-brown solid; yield: 92%; mp: 160 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.43 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.42–8.39 (m, 1H; NH), 8.32 (d, J = 8.05 Hz, 1H; quinoline H-5), 7.97 (s, 1H; quinoline H-8), 7.49–7.39 (m, 2H; quinoline H-6 and NH), 6.61 (d, J = 5.86 Hz, 1H; quinoline H-3), 4.13 (s, 2H; CH_2Cl), 3.42–3.27 ppm (m, 4H; 2×CH_2); IR (Nujol): ν_{max} = 3361 (NH), 3194 (NH), 1649 (C=O), 1584 (C=N), 1455, 1222, 1138, 897, 871, 849, 808, 762, 722 cm^{-1}; MS (ESI): m/z: 298 $[M+H]^+$.

2-Chloro-*N*-[3-(7-chloroquinolin-4-ylamino)propyl]acetamide (3b): Off-white solid; yield: 96%; mp: >200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.40 (d, J = 5.86 Hz, 1H; quinoline H-2), 8.37 (d, J = 5.13 Hz, 1H; NH), 8.29 (d, J = 9.52 Hz, 1H; quinoline H-5), 7.79 (d, J = 2.20 Hz, 1H; quinoline H-8), 7.48–7.46 (m, 2H; quinoline H-6 and NH), 6.49 (d, J = 5.13 Hz, 1H; quinoline H-3), 4.07 (s, 2H; CH_2Cl), 3.31 (q, J = 6.59 Hz, 2H; CH_2), 3.23 (q, J = 6.59 Hz, 2H; CH_2), 1.86–1.80 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.04, 151.09, 150.47, 139.06, 133.79, 126.68, 124.30, 124.23, 117.28, 98.63, 42.67, 40.11, 36.85, 27.50 ppm; IR (Nujol): ν_{max} = 3349 (NH), 3177 (NH), 1681 (C=O), 1613, 1582 (C=N), 1463, 1455, 1376, 1337, 1298, 1282, 1241, 1215, 1198, 1141, 1078, 1053, 935, 902, 854, 816, 799, 759, 722 cm^{-1}; MS (ESI): m/z: 312 $[M+H]^+$.

2-Chloro-*N*-[4-(7-chloroquinolin-4-ylamino)butyl]acetamide (3c): Dark-brown solid; yield: 90%; mp: >200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.36 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.32–8.30 (m, 2H; NH and quinoline H-5), 7.76 (s, 1H; quinoline H-8), 7.52 (d, J = 4.39 Hz, 1H; NH), 7.43–7.40 (m, 1H; quinoline H-6), 6.45 (d, J = 5.13 Hz, 1H; quinoline H-3), 4.03 (s, 2H; CH_2Cl), 3.26 (q, J = 5.86 Hz, 2H; CH_2), 3.13 (q, J = 5.86 Hz, 2H; CH_2), 1.67–1.50 ppm (m, 4H; 2×CH_2); IR (Nujol): ν_{max} = 3352 (NH), 3187 (NH), 1678 (C=O), 1576 (C=N), 1455, 1377, 1336, 1284, 1235, 1205, 1141, 1078, 954, 900, 849, 803, 722 cm^{-1}; MS (ESI): m/z: 326 $[M+H]^+$.

2-Chloro-*N*-[6-(7-chloroquinolin-4-ylamino)hexyl]acetamide (3d): Dark-brown solid; yield: 78%; mp: >200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.40 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.39 (d, J = 5.13 Hz, 1H; NH), 8.34 (d, J = 8.05 Hz, 1H; quinoline H-5), 7.77 (s, 1H; quinoline H-8), 7.51–7.41 (m, 2H; quinoline H-6 and NH), 6.54 (d, J = 5.13 Hz, 1H; quinoline H-3), 4.09 (s, 2H; CH_2Cl), 3.32–3.28 (m, 2H; CH_2), 3.11–3.06 (m, 2H; CH_2), 1.70–1.66 (m, 2H; CH_2), 1.52–1.34 ppm (m, 6H; 3×CH_2); IR (Nujol): ν_{max} = 3352 (NH), 3189 (NH), 1675 (C=O), 1587 (C=N), 1474, 1452, 1379, 1342, 1299, 1280, 1235, 1205, 1184, 942, 732 cm^{-1}; MS (ESI): m/z: 354 $[M+H]^+$.

General procedure for the synthesis of compounds 4a–d

Sodium azide (6.0 mmol) was added to a well stirred solution of compound (**3a–d**; 1.0 mmol) in MeOH (60 mL), and the resulting solution was heated at reflux for 7–8 h. After the completion of the reaction, the solvent was evaporated to dryness under vacuum. The obtained residue was dissolved in ethyl acetate (80 mL), and the solution was washed with water (40 mL×3), dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the desired products (**4a–d**) in good yields and in sufficiently pure forms to be used in the next step without further purification.

2-Azido-*N*-[2-(7-chloroquinolin-4-ylamino)ethyl]acetamide (4a): Yellow solid; yield: 79%; mp: 154 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.40 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.35 (br s, 1H; NH), 8.18 (d, J = 8.79 Hz, 1H; quinoline H-5), 7.78 (d, J = 2.20 Hz, 1H; quinoline H-8), 7.44 (d, J = 9.52 Hz, 1H; quinoline H-6), 7.39–7.38 (m, 1H; NH), 6.54 (d, J = 5.13 Hz, 1H; quinoline H-3), 3.86 (s, 2H; CH_2N_3), 3.37–3.35 ppm (m, 4H; 2×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 167.86, 151.93, 150.01, 149.07, 133.44, 127.53, 124.14, 123.94, 117.44, 98.62, 50.92, 41.85, 37.35 ppm; IR (Nujol): ν_{max} = 3368 (NH), 2113 (N_3), 1663 (C=O), 1581 (C=N), 1543, 1459, 1376, 1276, 1224, 1139, 1077, 899, 872, 849, 795, 760, 721 cm^{-1}; MS (ESI): m/z: 305 $[M+H]^+$.

2-Azido-*N*-[3-(7-chloro-quinolin-4-ylamino)propyl]acetamide (4b): Light-brown solid; yield: 84%; mp: 164 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.38 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.25 (d, J = 9.52 Hz, 1H; quinoline H-5), 8.24 (br s, 1H; NH), 7.78 (s, 1H; quinoline H-8), 7.42 (d, J = 8.79 Hz, 1H; quinoline H-6), 7.34 (s, 1H; NH), 6.44 (d, J = 5.13 Hz, 1H; quinoline H-3), 3.84 (s, 2H; CH_2N_3), 3.29–3.23 (m, 4H; 2×CH_2), 1.84–1.81 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 170.19, 154.43, 152.99, 151.58, 136.39, 130.07, 126.94, 126.86, 120.24, 101.46, 53.76, 42.84, 39.50, 30.48 ppm; IR (Nujol): ν_{max} = 3359 (NH), 3186 (NH), 2102 (N_3), 1693 (C=O), 1611, 1583 (C=N), 1546, 1456, 1425, 1374, 1337, 1280, 1253, 1216, 1141, 1079, 1054, 937, 901, 856, 814, 798, 757, 721, 644 cm^{-1}; MS (ESI): m/z: 319 $[M+H]^+$.

2-Azido-*N*-[4-(7-chloroquinolin-4-ylamino)butyl]acetamide (4c): Off-white solid; yield: 91%; mp: 168 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.36 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.25 (d, J = 8.79 Hz, 1H; quinoline H-5), 8.11 (t, J = 5.13 Hz, 1H; NH), 7.76 (d, J = 2.20 Hz, 1H; quinoline H-8), 7.41 (dd, J = 8.79, 2.20 Hz, 1H; quinoline H-6), 7.30 (t, J = 5.13 Hz, 1H; NH), 6.44 (d, J = 5.86 Hz, 1H; quinoline H-3), 3.78 (s, 2H; CH_2N_3), 3.25 (q, J = 6.59 Hz, 2H; CH_2), 3.14 (q, J = 6.59 Hz, 2H; CH_2), 1.66–1.60 (m, 2H; CH_2), 1.56–1.50 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 167.20, 151.91, 150.12, 149.08, 133.43, 127.46, 124.12, 124.03, 117.47, 98.68, 50.85, 42.04, 38.39, 26.69, 25.17 ppm; IR (Nujol): ν_{max} = 3376 (NH), 3184 (NH), 2120 (N_3), 1667 (C=O), 1586 (C=N), 1463, 1455, 1373, 1335, 1283, 1255, 1208, 1141, 1083, 998, 917, 901, 853, 814, 793, 761, 722, 644 cm^{-1}; MS (ESI): m/z: 333 $[M+H]^+$.

2-Azido-*N*-[6-(7-chloroquinolin-4-ylamino)hexyl]acetamide (4d): Dark-brown solid; yield: 88%; mp: 124 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.40 (d, J = 5.13 Hz, 1H; quinoline H-2), 8.31 (d, J = 8.79 Hz, 1H; quinoline H-5), 8.13 (t, J = 5.13 Hz, 1H; NH), 7.80 (d, J = 2.20 Hz, 1H; quinoline H-8), 7.44 (dd, J = 8.79, 2.20 Hz, 1H; quinoline H-6), 7.36 (br s, 1H; NH), 6.45 (d, J = 5.13 Hz, 1H; quinoline H-3), 3.81 (s, 2H; CH_2N_3), 3.28–3.23 (m, 2H; CH_2), 3.13–3.08 (m, 2H; CH_2), 1.68–1.62 (m, 2H; CH_2), 1.48–1.30 ppm (m, 6H; 3×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 167.04, 151.64, 150.22, 148.82, 133.47, 127.22, 124.15, 123.99, 117.40, 98.54, 50.83, 42.36, 38.59, 28.94, 27.71, 26.32, 26.16 ppm; IR (Nujol): ν_{max} = 3369 (NH), 3191 (NH), 2105 (N_3), 1681 (C=O), 1609, 1576 (C=N), 1534, 1463, 1376, 1327, 1284, 1250, 1167, 1134, 1078, 905, 870, 854, 815, 798, 722 cm^{-1}; MS (ESI): m/z: 361 $[M+H]^+$.

General procedure for the synthesis of compounds 7, 8a,b, 9a–e, 10a,b and 11a,b

Alkyne (**5** or **6a–e**; 3.5 mmol), $CuSO_4$·5H_2O (1.0 mmol) and sodium ascorbate (2.2 mmol) were added to a solution of azide (**4a–d**; 3.0 mmol) in 50% *t*-butanol in water (20 mL). The reaction mixture was stirred at room temperature for 8 h. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (80 mL) and the product was extracted with ethyl acetate (60 mL×3). The ethyl acetate layers were combined, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The obtained crude product was stirred in diethyl ether (60 mL) at room

temperature for 2 h. The solid was then filtered, washed well with diethyl ether and dried under vacuum to obtain the pure products in good to excellent yields.

***N*-[3-(7-Chloroquinolin-4-ylamino)propyl]-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetamide (7)**: Pale-yellow solid; yield: 87%; mp: >200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.47 (brs, 3H; NH, quinoline H-2 and quinoline H-5), 8.22 (s, 1H; triazole H), 7.81–7.76 (m, 3H; ArH and quinoline H-8), 7.41–7.29 (m, 5H; ArH, quinoline H-6 and NH), 6.47 (s, 1H; quinoline H-3), 5.11 (s, 2H; CH_2CO), 3.45–3.22 (m, 4H; 2×CH_2), 1.86–1.80 ppm (m, 2H; CH_2); IR (Nujol): ν_{max} = 3273 (NH), 1654 (C=O), 1583 (C=N), 1455, 1377, 1079, 976, 846, 805, 762, 721, 689 cm^{-1}; MS (ESI): *m/z*: 421 $[M+H]^+$.

2-[4-(4-Chlorophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[2-(7-chloroquinolin-4-ylamino)ethyl]acetamide (8a): White solid; yield: 85%; mp: 190 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.58 (s, 1H; quinoline H-2), 8.43 (brs, 1H; NH), 8.21 (s, 1H; quinoline H-5), 8.19 (s, 1H; triazole H), 7.81 (s, 1H; quinoline H-8), 7.47(d, *J* = 8.79 Hz, 1H; quinoline H-6), 7.40 (s, 1H; NH), 7.35 (d, *J* = 8.79 Hz, 2H; ArH), 7.08 (d, *J* = 8.79 Hz, 2H; ArH), 6.57 (d, *J* = 4.39 Hz, 1H; quinoline H-3), 5.16 (s, 2H; CH_2O), 5.15 (s, 2H; CH_2CO), 3.40–3.37 ppm (m, 4H; 2×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.97, 156.90, 151.93, 150.01, 149.07, 142.14, 133.46, 129.25, 127.54, 126.21, 124.53, 124.18, 123.98, 123.86, 116.46, 98.67, 61.28, 51.64, 41.73, 37.46 ppm; IR (Nujol): ν_{max} = 3385 (NH), 3272 (NH), 1660 (C=O), 1574 (C=N), 1455, 1377, 1239, 1170, 1137, 1077, 1041, 903, 880, 855, 812, 787, 722, 652 cm^{-1}; MS (ESI): *m/z*: 471 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{20}Cl_2N_6O_2\cdot0.2H_2O$: C 55.64, H 4.33, N 17.69; found: C 55.57, H 4.45, N 17.57.

2-[4-(4-Bromophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[2-(7-chloroquinolin-4-ylamino)ethyl]acetamide (8b): Off-white solid; yield: 87%; mp: 184 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.58 (s, 1H; quinoline H-2), 8.43 (brs, 1H; NH), 8.21 (d, *J* = 8.79 Hz, 1H; quinoline H-5), 8.19 (s, 1H; triazole H), 7.81 (s, 1H; quinoline H-8), 7.48–7.45 (m, 4H; ArH, quinoline H-6 and NH), 7.04 (d, *J* = 8.79 Hz, 2H; ArH), 6.58 (brs, 1H; quinoline H-3), 5.16 (s, 2H; CH_2O), 5.15 (s, 2H; CH_2CO), 3.43–3.40 ppm (m, 4H; 2×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.98, 157.34, 151.72, 150.11, 142.13, 133.54, 132.15, 132.03, 131.97, 127.43, 126.23, 124.25, 124.05, 117.01, 112.27, 98.87, 61.21, 51.64, 39.91, 39.29 ppm; IR (Nujol): ν_{max} = 3387 (NH), 3273 (NH), 1654 (C=O), 1582 (C=N), 1458, 1376, 1237, 1172, 1139, 1054, 811, 721 cm^{-1}; MS (ESI): *m/z*: 516 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{20}BrClN_6O_2\cdot0.6H_2O$: C 50.18, H 4.06, N 15.96; found: C 50.18, H 3.92, N 15.64.

***N*-[3-(7-Chloroquinolin-4-ylamino)propyl]-2-(4-phenoxymethyl-1,2,3-triazol-1-yl)acetamide (9a)**: Pale-yellow solid; yield: 78%; mp: 174 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.47 (brs, 2H; quinoline H-2 and NH), 8.27 (d, *J* = 8.05 Hz, 1H; quinoline H-5), 8.18 (s, 1H; triazole H), 7.80 (s, 1H; quinoline H-8), 7.45 (d, *J* = 8.79 Hz, 1H; quinoline H-6), 7.32 (brs, 1H; NH), 7.29 (d, *J* = 7.32 Hz, 2H; ArH), 7.04 (d, *J* = 8.79 Hz, 2H; ArH), 6.95 (t, *J* = 7.32, 1H; ArH), 6.50 (brs, 1H; quinoline H-3), 5.14 (s, 2H; CH_2O), 5.13 (s, 2H; CH_2CO), 3.32 (t, *J* = 6.59 Hz, 2H; CH_2), 3.25 (t, *J* = 6.59 Hz, 2H; CH_2), 1.86–1.83 ppm (m, 2H; CH_2); IR (Nujol): ν_{max} = 3369 (NH), 1693 (C=O), 1574 (C=N), 1539, 1488, 1455, 1377, 1332, 1281, 1241, 1177, 1141, 1118, 1098, 1079, 1058, 1036, 942, 893, 864, 848, 820, 797, 767, 751, 722, 691, 644, 621 cm^{-1}; MS (ESI): *m/z*: 451 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{23}ClN_6O_2\cdot0.8H_2O\cdot0.1C_4H_{10}O$: C 59.45, H 5.46, N 17.78; found: C 59.41, H 5.54, N 17.44.

2-[4-(4-Chlorophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[3-(7-chloroquinolin-4-ylamino)propyl]acetamide (9b): Pale-yellow solid; yield: 82%; mp: 192 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.47 (s, 1H; quinoline H-2), 8.42 (brs, 1H; NH), 8.26 (d, *J* = 8.79 Hz, 1H; quinoline H-5), 8.19 (s, 1H; triazole H), 7.80 (s, 1H; quinoline H-8), 7.45 (d, *J* = 8.79 Hz, 1H; quinoline H-6), 7.35 (s, 1H; NH), 7.33–7.31 (m, 2H; ArH), 7.08 (d, *J* = 9.52 Hz, 2H; ArH), 6.49 (d, *J* = 4.39 Hz, 1H; quinoline H-3), 5.16 (s, 2H; CH_2O), 5.14 (s, 2H; CH_2CO), 3.32 (t, *J* = 6.59 Hz, 2H; CH_2), 3.25 (t, *J* = 6.59 Hz, 2H; CH_2), 1.88–1.81 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.44, 156.90, 151.93, 150.03, 149.06, 142.11, 133.43, 129.25, 127.51, 126.25, 124.53, 124.11, 124.08, 117.48, 116.47, 98.78, 61.27, 51.69, 39.91, 36.83, 27.63 ppm; IR (Nujol): ν_{max} = 3379 (NH), 1678 (C=O), 1570 (C=N), 1542, 1518, 1469, 1365, 1329, 1285, 1255, 1182, 1158, 1065, 946, 896, 877, 848, 820, 799 cm^{-1}; MS (ESI): *m/z*: 486 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{22}Cl_2N_6O_2\cdot0.89H_2O$: C 55.10, H 4.78, N 16.76; found: C 54.93, H 4.45, N 16.42.

2-[4-(4-Bromophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[3-(7-chloroquinolin-4-ylamino)propyl]acetamide (9c): Pale-yellow solid; yield: 77%; mp: 202 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.45 (d, *J* = 5.13 Hz, 1H; quinoline H-2), 8.43 (s, 1H; NH), 8.26 (d, *J* = 8.79 Hz, 1H; quinoline H-5), 8.18 (s, 1H; triazole H), 7.79 (s, 1H; quinoline H-8), 7.46 (dd, *J* = 6.59, 2.20 Hz, 2H; ArH), 7.45–7.44 (m, 1H; quinoline H-6), 7.31 (t, *J* = 5.13 Hz, 1H; NH), 7.03 (dd, *J* = 7.69, 2.20 Hz, 2H; ArH), 6.49 (d, *J* = 4.39, Hz, 1H; quinoline H-3), 5.15 (s, 2H; CH_2O), 5.12 (s, 2H; CH_2CO), 3.31 (t, *J* = 6.59 Hz, 2H; CH_2), 3.24 (t, *J* = 6.59 Hz, 2H; CH_2), 1.87–1.80 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 168.10, 160.00, 154.49, 152.69, 150.23, 149.55, 144.75, 136.11, 134.87, 130.15, 128.91, 126.79, 126.75, 119.67, 114.93, 98.55, 63.87, 54.35, 42.77, 39.50, 30.29 ppm; IR (Nujol): ν_{max} = 3364 (NH), 1688 (C=O), 1574 (C=N), 1538, 1463, 1455, 1377, 1332, 1300, 1281, 1242, 1171, 1141, 1118, 1099, 1074, 1045, 1028, 945, 894, 871, 848, 823, 797, 766, 722, 643, 621 cm^{-1}; MS (ESI): *m/z*: 530 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{22}BrClN_6O_2\cdot0.3H_2O$: C 51.61, H 4.26, N 15.70; found: C 51.73, H 4.53, N 15.38.

***N*-[3-(7-Chloroquinolin-4-ylamino)propyl]-2-(4-*p*-tolyloxymethyl-1,2,3-triazol-1-yl)acetamide (9d)**: Light-brown solid; yield: 79%; mp: 196 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.46 (brs, 2H; quinoline H-2 and H-5), 8.17 (s, 1H; triazole H), 7.95–7.87 (brs, 2H; NH and quinoline H-8), 7.45 (d, *J* = 8.79 Hz, 1H; quinoline H-6), 7.38 (s, 1H; NH), 7.09 (d, *J* = 8.05 Hz, 2H; ArH), 6.92 (d, *J* = 8.79 Hz, 2H; ArH), 6.66 (brs, 1H; quinoline H-3), 5.13 (s, 2H; CH_2O), 5.10 (s, 2H; CH_2CO), 3.30–3.25 (m, 4H; 2×CH_2), 2.23 (s, 3H; CH_3), 1.86–1.83 ppm (m, 2H; CH_2); IR (Nujol): ν_{max} = 3371 (NH), 1689 (C=O), 1576 (C=N), 1540, 1513, 1455, 1377, 1332, 1282, 1247, 1175, 1141, 1059, 944, 895, 874, 849, 822, 798, 765, 722 cm^{-1}; MS (ESI): *m/z*: 465 $[M+H]^+$; elemental analysis calcd (%) for $C_{24}H_{25}ClN_6O_2\cdot H_2O$: C 59.69, H 5.63, N 17.40; found: C 59.77, H 5.91, N 17.09.

***N*-[3-(7-Chloroquinolin-4-ylamino)propyl]-2-{4-[(4-formylphenoxy)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide (9e)**: Pale-yellow solid; yield: 88%; mp: 182 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.86 (s, 1H; CHO), 8.44–8.42 (m, 2H; quinoline H-2 and NH), 8.25 (d, *J* = 9.52 Hz, 1H; quinoline H-5), 8.21 (s, 1H; triazole H), 7.87 (d, *J* = 8.79 Hz, 2H; ArH), 7.77 (s, 1H; quinoline H-8), 7.45 (d, *J* = 8.79 Hz, 1H; quinoline H-6), 7.35 (t, *J* = 5.13 Hz, 1H; NH), 7.24 (d, *J* = 8.79 Hz, 2H; ArH), 6.49 (d, *J* = 4.39 Hz, 1H; quinoline H-3), 5.28 (s, 2H; CH_2O), 5.12 (s, 2H; CH_2CO), 3.26–3.21 (m, 4H; 2×CH_2), 1.86–1.81 ppm (m, 2H; CH_2); IR (Nujol): ν_{max} = 3274 (NH), 1686 (C=O), 1656 (CHO), 1599 (C=N), 1508, 1459, 1376, 1262, 1217, 1162, 1141, 1052, 1032, 999, 944, 902, 827, 765, 721 cm^{-1}; MS (ESI): *m/z*: 479 $[M+H]^+$.

2-[4-(4-Chlorophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[4-(7-chloroquinolin-4-ylamino)butyl]acetamide (10a): Pale-yellow solid;

yield: 76%; mp: 198 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.41–8.38 (m, 3H; quinoline H-2, H-5 and NH), 8.18 (s, 1H; triazole H), 7.85 (s, 1H; quinoline H-8), 7.45 (d, J = 9.52 Hz, 1H; quinoline H-6), 7.38 (s, 1H; NH), 7.34 (d, J = 8.05 Hz, 2H; ArH), 7.07 (d, J = 8.79 Hz, 2H; ArH), 6.56 (s, 1H; quinoline H-3), 5.15 (s, 2H; CH_2O), 5.10 (s, 2H; CH_2CO), 3.28–3.27 (m, 2H; CH_2), 3.20–3.17 (m, 2H; CH_2), 1.68–1.66 (m, 2H; CH_2), 1.58–1.54 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.23, 156.90, 151.76, 150.12, 144.51, 142.08, 133.45, 129.26, 127.52, 127.42, 126.24, 124.53, 124.19, 124.10, 116.47, 98.21, 61.26, 51.64, 42.03, 38.52, 26.61, 25.16 ppm; IR (Nujol): ν_{max} = 3278 (NH), 1654 (C=O), 1611, 1582 (C=N), 1489, 1463, 1377, 1283, 1243, 1217, 1170, 1139, 1093, 1056, 1033, 1005, 899, 851, 819, 803, 765, 722, 644 cm^{-1}; MS (ESI): m/z: 500 $[M+H]^+$; elemental analysis calcd (%) for $C_{24}H_{24}Cl_2N_6O_2 \cdot 1.4H_2O$: C 54.95, H 5.15, N 16.02; found: C 55.15, H 4.93, N 15.63.

***N*-[4-(7-Chloroquinolin-4-ylamino)butyl]-2-(4-*p*-tolyloxymethyl-1,2,3-triazol-1-yl)acetamide (10b)**: White solid; yield: 82%; mp: 202 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.37 (br s, 2H; NH and quinoline H-2), 8.27 (d, J = 7.32 Hz, 1H; quinoline H-5), 8.13 (s, 1H; triazole H), 7.78 (s, 1H; quinoline H-8), 7.42 (d, J = 8.79 Hz, 1H; quinoline H-6), 7.31 (s, 1H; NH), 7.07 (d, J = 8.05 Hz, 2H; ArH), 6.90 (d, J = 8.05 Hz, 2H; ArH), 6.47 (s, 1H; quinoline H-3), 5.08 (s, 4H; CH_2O and CH_2CO), 3.27–3.25 (m, 2H; CH_2), 3.17–3.15 (m, 2H; CH_2), 2.21 (s, 3H; CH_3), 1.66–1.54 ppm (m, 4H; 2×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.24, 155.95, 151.82, 150.01, 142.55, 133.34, 129.82, 129.69, 129.44, 125.99, 124.16, 124.02, 114.46, 114.29, 98.52, 60.94, 51.62, 42.00, 38.51, 26.61, 25.16, 20.07 ppm; IR (Nujol): ν_{max} = 3300 (NH), 1651 (C=O), 1612, 1584 (C=N), 1543, 1508, 1463, 1455, 1377, 1330, 1252, 1235, 1211, 1171, 1138, 1081, 1059, 1035, 1007, 901, 876, 853, 818, 803, 766, 721, 645 cm^{-1}; MS (ESI): m/z: 479 $[M+H]^+$.

2-[4-(4-Chlorophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[6-(7-chloroquinolin-4-ylamino)hexyl]acetamide (11a): White solid; yield: 79%; mp: 116 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.35–8.32 (m, 2H; quinoline H-2 and NH), 8.25 (d, J = 8.79 Hz, 1H; quinoline H-5), 8.15 (s, 1H; triazole H), 7.76 (s, 1H; quinoline H-8), 7.41 (dd, J = 9.15, 2.20 Hz, 1H; quinoline H-6), 7.32–7.29 (m, 3H; ArH and NH), 7.04 (dd, J = 7.32, 2.20 Hz, 2H; ArH), 6.44 (d, J = 5.13 Hz, 1H; quinoline H-3), 5.12 (s, 2H; CH_2O), 5.07 (s, 2H; CH_2CO), 3.23 (q, J = 6.59 Hz, 2H; CH_2), 3.08 (q, J = 6.59 Hz, 2H; CH_2), 1.65–1.61 (m, 2H; CH_2), 1.44–1.31 ppm (m, 6H; 3×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 162.28, 159.50, 154.03, 148.96, 147.30, 146.10, 139.21, 126.39, 124.50, 123.37, 121.67, 121.28, 121.18, 114.57, 113.60, 95.77, 58.40, 48.78, 39.50, 35.89, 26.04, 24.87, 23.46, 23.30 ppm; IR (Nujol): ν_{max} = 3284 (NH), 1654 (C=O), 1581 (C=N), 1458, 1376, 1280, 1243, 1168, 1138, 1094, 1056, 1004, 899, 848, 821, 722, 643 cm^{-1}; MS (ESI): m/z: 528 $[M+H]^+$; elemental analysis calcd (%) for $C_{26}H_{28}Cl_2N_6O_2 \cdot 0.7H_2O$: C 57.82, H 5.49, N 15.56; found: C 57.82, H 5.56, N 15.27.

2-[4-(4-Bromophenoxymethyl)-1,2,3-triazol-1-yl]-*N*-[6-(7-chloroquinolin-4-ylamino)hexyl]acetamide (11b): White solid; yield: 82%; mp: 186 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.36–8.33 (m, 2H; NH and quinoline H-2), 8.26 (d, J = 8.79 Hz, 1H; quinoline H-5), 8.16 (s, 1H; triazole H), 7.76 (s, 1H; quinoline H-8), 7.44–7.42 (m, 3H; ArH and quinoline H-6), 7.31 (s, 1H; NH), 7.00 (d, J = 8.05 Hz, 2H; ArH), 6.44 (d, J = 4.39 Hz, 1H; quinoline H-3), 5.12 (s, 2H; CH_2O), 5.07 (s, 2H; CH_2CO), 3.24–3.22 (m, 2H; CH_2), 3.09–3.08 (m, 2H; CH_2), 1.63–1.34 ppm (m, 8H; 4×CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.54, 157.75, 152.19, 150.59, 149.33, 142.45, 133.86, 132.55, 127.75, 126.64, 124.56, 124.46, 117.84, 117.42, 112.66, 99.03, 61.62, 52.06, 42.77, 39.16, 29.32, 28.14, 26.74, 26.57 ppm; IR (Nujol): ν_{max} = 3281 (NH), 1655 (C=O), 1611, 1581 (C=N), 1462, 1377, 1282, 1244, 1170, 1140, 1074, 1055, 1032, 1001, 898, 848, 818, 722 cm^{-1}; MS (ESI): m/z: 572 $[M+H]^+$.

Biological assays

Culture adaptation and in vitro cultivation of asexual stages: Cryo-preserved *P. falciparum* strains (CQ-sensitive 3D7 and CQ-resistant RKL9, a field isolate collected from Rourkela, India) were revived according to standard protocols and introduced into culture with the objective of their adaptation to in vitro conditions. Asexual stages were cultivated by following the procedures of Trager and Jensen[31,32] with minor modifications. The parasites were cultivated in RPMI-1640 medium (with glutamine; GIBCO) containing HEPES (25 mM), D-glucose (2 g L^{-1}), sodium bicarbonate (2 g L^{-1}), and gentamicin sulfate (40 μg mL^{-1}), supplemented with 10% heat-inactivated AB$^+$ human serum. A$^+$ human blood at 10% haematocrit was used as a source of host erythrocytes. Cultures were maintained in an incubator at 37 °C in a 5% CO_2 atmosphere. Synchronised parasites at ring stages were obtained by treatment with 5% sorbitol (*w/v*) as required.

In vitro production of gametocytes: The procedures used for gametocyte production are described in detail elsewhere.[33] In brief, on the day of initiating the gametocyte culture (day 0), an asexual culture was synchronised with sorbitol to obtain >90% ring stages, and parasitaemia was lowered to 0.5% by addition of fresh erythrocytes (10% haematocrit). These parasites were replenished daily with complete RPMI-1640 media supplemented with hypoxanthine (50 μg mL^{-1}) and kept free of fresh erythrocytes throughout the period of culture maintenance. Haematocrit was reduced to 5% on day 8 and *N*-acetylglucosamine (50 mM) was added on days 9–12 to eliminate asexual stages and enrich for gametocytes. On day 14 onwards, a gametocyte culture with a majority of mature gametocytes (stages IV and V) was obtained and used for evaluating the compounds.

Selection of the most potent compounds using a malaria SYBR Green I fluorescence assay: All 12 quinoline–triazole hybrids were initially screened for their antimalarial activity using an MSF assay[34] to select the most potent compounds for a detailed investigation. Appropriate stock solutions of compounds and the standard drug CQ were prepared in DMSO (final DMSO concentrations <0.5% *v/v*) and water, respectively, and stored at −20 °C until use. Appropriate working solutions were freshly prepared with complete culture medium on the day of the experiment. Twofold serial dilutions of test samples were prepared in triplicate in 96-well microtiter plates and incubated with 1% parasitised red blood cell suspension having 0.8–1% parasitaemia (>90% rings, sorbitol synchronised). Positive and negative control wells without the test compounds were also prepared, with 1% parasitised and non-parasitised erythrocyte suspension, respectively. Unused parasite culture was kept separately without drug for monitoring parasite growth. Plates were incubated in a 5% CO_2 atmosphere at 37 °C for 72 h. After this incubation period, lysis buffer (Tris, EDTA, saponin, Triton X-100, 2× concentration of SYBR Green I (Invitrogen) in water, 100 μL) was added to each well and incubated in the dark for 1 h at 37 °C. The relative fluorescence units (RFUs) per well were measured using a fluorescence plate reader at excitation and emission wavelengths of 485±20 and 530±20 nm, respectively. The half-maximal inhibitory concentration values (IC_{50}) for each compound were determined using a nonlinear regression analysis of dose–response curves generated using a pre-programmed Microsoft Excel spread sheet after two independent experiments.

Evaluation of antimalarial activity against chloroquine-resistant *P. falciparum*: Selected compounds that showed promising activity against 3D7 were further tested for their antimalarial potential against a CQ-resistant field isolate (RKL-9) of *P. falciparum* using a schizont maturation inhibition assay.[35–37] In brief, for carrying out dose–response evaluation, twofold dilutions of test compounds at the desired concentrations were prepared in 96-well microtiter plates. Inhibition experiments were typically carried out at a final concentration of 4 μM. Sorbitol-synchronised parasites with 1% parasitaemia (>90% rings) were added to each well at the start of experiment. Dihydroartemisinin (100 nM) and 0.5% DMSO (*v*/*v*) were used as positive and negative (vehicle) controls, respectively. Separate wells without test compounds/drugs were used to monitor uninhibited growth. Microtiter plates were incubated at 37 °C in the presence of 5% CO_2 for 22–36 h, until >10% schizont maturation (schizonts with four or more merozoites) in untreated wells was observed. At the end of the incubation period, thin smears were prepared from each well and stained with 10% Giemsa for 20 min. Schizont maturation was assessed by counting number of schizonts per 200 asexual parasites. Schizont maturation data collected for each treated well was compared with data obtained from untreated control wells and collectively used to calculate the percentage inhibition. Percentage inhibition data was plotted against the log of concentration using a nonlinear regression analysis with four-parameter log dose with variable slope to compute IC_{50} values and 95% confidence intervals (Table 1) using GraphPad Prism 6. Compounds were tested in three independent experiments.

Gametocytocidal assays and data analysis: Gametocytocidal drug assays were carried out in three independent experiments and potency was evaluated as described elsewhere.[33] In brief, gametocytes with a majority of late stages were harvested on the day of experiment and a systematic morphological examination was performed by light microscopy before carrying out the screening experiments. Test plates were prepared by plating twofold dilutions of the drugs in duplicates to achieve concentrations of up to 50 μM for test compounds and then incubated with blood containing 2–3% mature gametocytes. Control wells were also prepared containing gametocytes with drug-free media for evaluation of untreated inhibition. Also, 0.5% DMSO and methylene blue (10 μM) were used as vehicle (negative) and positive controls, respectively. Plates were incubated in a 5% CO_2 atmosphere at 37 °C for 48 h.[38] After incubation period, thin smears were prepared, stained with 10% Giemsa and examined under a 100× oil immersion objective.[39] Five thousand erythrocytes from each smear were counted to examine gametocytaemia and gametocyte morphology at each concentration. Late stage gametocytes (stages IV and V) observed were categorised into two morphological groups, 1) the normal morphology (NM) group containing healthy gametocytes and 2) the altered morphology (AM) group containing morphologically deformed/unhealthy gametocytes. Gametocytaemia for each concentration was expressed as the percentage inhibition compared to drug-free control, which was plotted against the log of concentration using a nonlinear regression analysis (four-parameter log dose with variable slope) to calculate IC_{50} values and 95% confidence intervals. Dose–response curves expressed as percentage inhibition versus logarithm of drug concentration were generated by GraphPad Prism 6. IC_{50} values were also calculated in two categories. In the first category, only gametocytes bearing normal morphology were included and the activity was termed "IC_{50} (NM)". In the second category, gametocytes with both normal and altered morphology were included and IC_{50} is designated as "IC_{50} (total)".

Cytotoxicity studies: Cytotoxicity evaluation was carried out against VERO cells (C 1008; monkey kidney fibroblast) using the MTT cell viability assay by following published protocols with minor modifications.[40] In brief, the desired concentration ranges of compounds were plated in 96-well microtiter plate after serially diluting the compound solutions. Cells were seeded with the compound dilutions and incubated in a 5% CO_2 atmosphere at 37 °C for 72 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg mL^{-1}) reagent was added to each well and plates were incubated in the dark for 4 h. The by-product (dark-blue formazan) formed by viable cells was dissolved in DMSO and the absorbance was recorded at 550 nm using an ELISA reader. 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis. SI was calculated as the CC_{50}/IC_{50} ratio for each compound using the 3D7 strain of *P. falciparum*.

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Conflict of interest

The authors declare no conflict of interest.

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