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

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Abstract: Novel 4-amino-7-chloroquinoline based [1,2,3]-triazole hybrids were synthesized in good yields via Cu[I] catalysed Huisgen 1,3-dipolar cycloaddition reaction 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 chloroquine sensitive 3D7 strain of *P. falciparum*. The most active compounds were further screened against asexual and sexual stages (gametocytes) of chloroquine resistant RKL-9 strain of *P. falciparum*. Although all compounds were less potent than chloroquine against 3D7 strain, the three best compounds **8a**, **8b** and **9c** were appreciably more active than chloroquine against the RKL-9 strain, displaying IC_{50} values of <100 nM, with **8b** showing 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. These 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 tools. 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 our fight against this deadly disease, whether as chemo-prophylactic agents or in the treatment regimen. However, our malaria elimination efforts have been jeopardized 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 absence 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 the ones with transmission blocking potential is extremely crucial to tackle the ongoing threat of drug resistance and to curb malaria transmission.[4] In order to widen the scope of treatment and support malaria elimination program of the World Health Organization, developing antimalarial drugs having broader therapeutic potential which can simultaneously target both asexual blood stages that cause disease symptoms and gametocytes, stages responsible for transmission is beneficial. Historically, the most targeted pathway for antimalarial drug development is detoxification of heme and formation of hemozoin.[5] A number of highly effective 4-aminoquinoline antimalarial drugs such as CQ and amodiaquine (AQ) function by targeting this pathway. Structure-activity studies of 4-aminoquinolines have suggested that the presence of chlorine group at seventh position and amino group at terminal position is required for antimalarial activity of quinolines 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 improvised antimalarials with high potency against *P. falciparum.*[9] Moreover, studies on 4-aminoquinoline analogues also resulted in generation of promising lead compounds[10-12] and several candidates are in preclinical development or clinical trials.[13] Considering safety and efficacy of 4-aminoquinoline based drugs and remarkable success of CQ in the past, 4-aminoquinoline base is pharmaceutically suitable for use as a scaffold to develop new antimalarial drug candidates.

However, synthesis of compounds solely based on this 4-aminoquinoline moiety might exhibit similar cross resistance susceptibility patterns as shared by other established 4-aminoquinolines such as CQ and AQ and might illicit drug resistance responses earlier in areas with reported 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 another pharmacologically significant class of compounds known as [1,2,3]-triazoles, appended with 7-chloro-4-aminoquinoline base. These [1,2,3]-triazoles, exhibit myriad of biological activities including antifungal,[14] antibacterial[15] and antitubercular activities.[16] Apart from just being a passive linker, the triazoles can also act as important constituent of antimalarial compounds[17] because they themselves are lipophilic nitrogen

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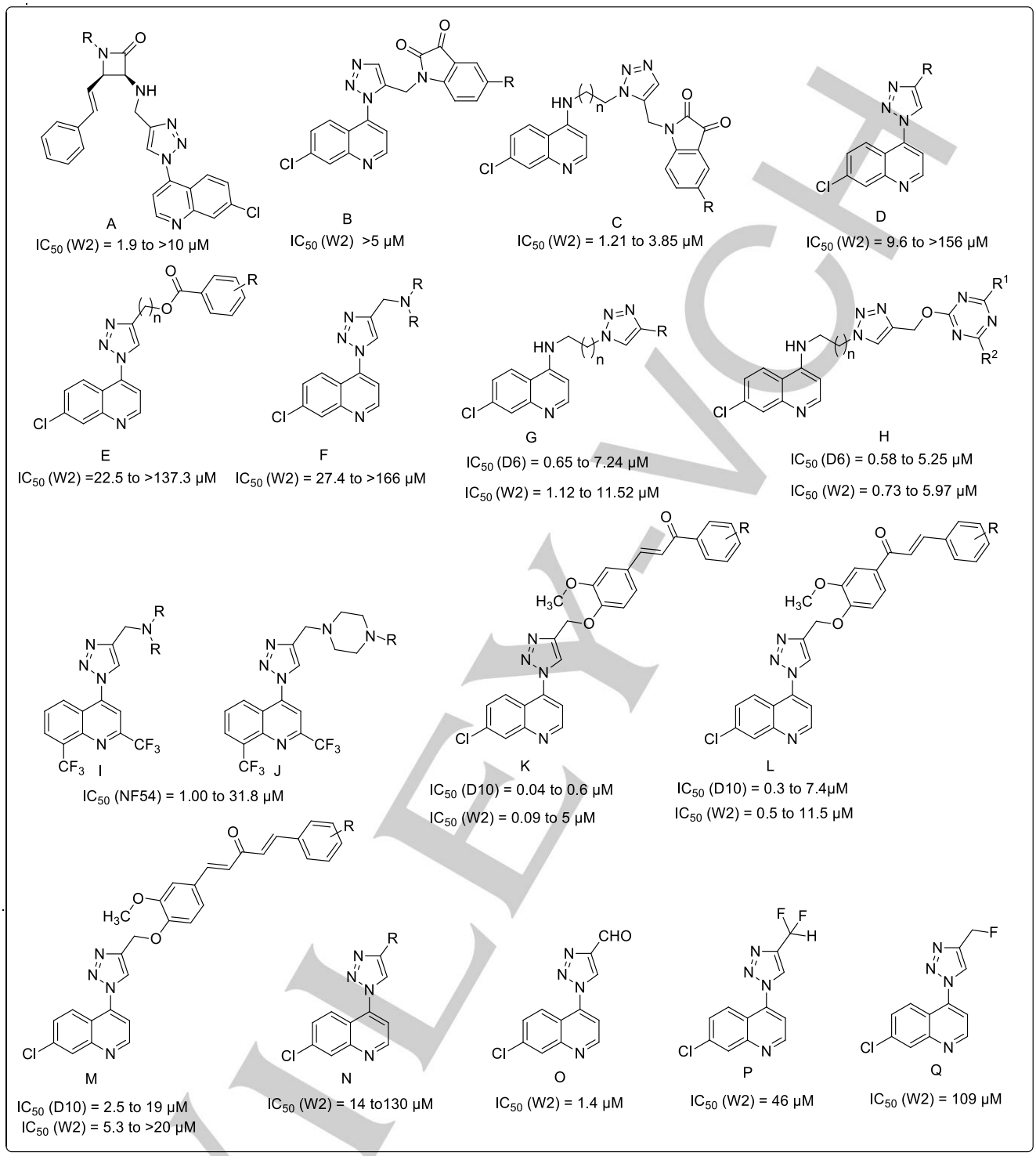


Figure 1. Prototypes and IC_{50} values of quinoline and triazole based hybrids reported by Singh et al.[19] (A), Raj et al.[20] (B and C), Pereira et al.[21] (D to F), Rawat et al.[22] (G and H), Hamann et al.[23] (I to J), Guantai et al.[24] (K to M) and Boechat et al.[25] (N to Q). For the nature of R in the prototypes and other details, please refer to the respective references.

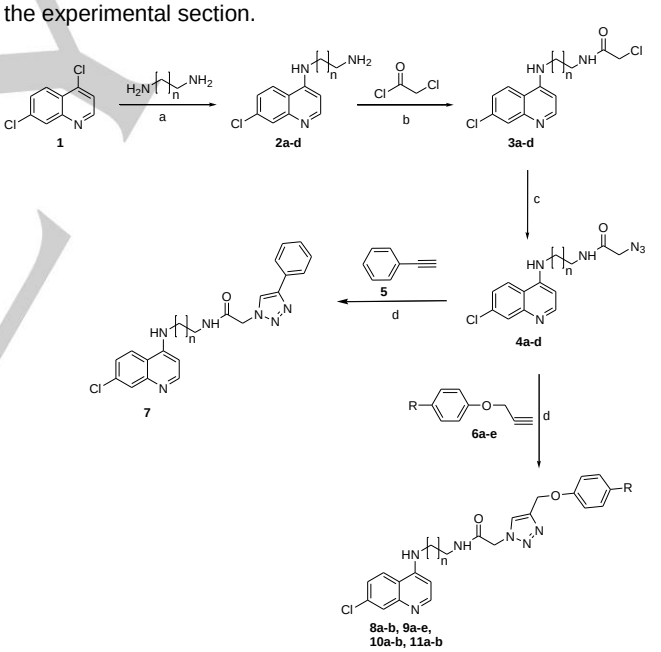
containing heterocycles that tend to accumulate into the food vacuole of the parasite, a property also expressed by CQ.[18] Prototypes of reported quinoline based triazole hybrids (A to Q) synthesized by other investigators along with their antimalarial activity in terms of IC_{50} are depicted in **Figure 1**. [1,2,3]-Triazole tethered 7-chloroquinoline and β-lactam bifunctional hybrids where [1,2,3]-triazole acted as a linker between β-lactam ring (connected at 4th position of triazole via amino methyl group) and 7-chloroquinoline moiety, demonstrated IC_{50} values in the range of 1.9 μM to >10 μM for chloroquine 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 when [1,2,3]-triazole is linked to 7-chloroquinoline skeleton by an aliphatic amine linker as compared to the hybrids where the [1,2,3]-triazole is covalently bonded to 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 W2 strain of *P. falciparum*.[20] Further, synthesis via copper-catalysed cycloaddition reaction was carried out to yield hybrids with [1,2,3]-triazole moiety (containing different substituents) directly attached to 7-chloroquinoline base. These compounds displayed potency in the range of 9.6 µM to >166 µM against W2 strain of *P. falciparum* (Figure-1; structures D to F).[21] Rawat et al. evaluated 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) where triazole moiety is appended to the 7-chloro-4-aminoquinoline base by an aliphatic linker. Here, IC_{50} values for 4-amino-7-chloroquinoline-[1,2,3]-triazole and 4-amino-7-chloroquinoline-[1,2,3]-triazole-triazine hybrids were reported to be in range (0.65 µM to 7.24 µM) and (0.58 µM to 5.25 µM) for CQ sensitive D6 strain, respectively and (1.12 µM to 11.52 µM) and (0.73 µM to 5.97 µM) for CQ resistant W2 strain, respectively.[22] The concept of triazole as a linker between two moieties was further escalated to synthesis of compounds with mefloquine (Figure 1; structures I and J) instead of the standard 7-chloroquinoline and IC_{50} values were reported to be in range (1.00 µM to 31.8 µM) for NF54 strain of *P. falciparum*.[23] Guantai and co-workers developed antimalarial compounds (Figure 1; structures K to M) where [1,2,3]-triazoles acted as a linker between a chalcone functionality (connected at 4th position of triazole via ether linkage) and 7-chloroquinoline moiety with IC_{50} values between (0.04 µM to 19 µM) for CQ sensitive D10 strain and (0.09 µM to >20 µM) for 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 4th position of 1,2,3-triazole ring were synthesized and half maximal inhibitory concentration was reported to be in range (1.4 µM to 130 µM) against CQ resistant W2 strain of *P. falciparum*.[25] In majority of these studies, 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] Fascinated by the previously reported work, and in consensus with our aim to develop novel antimalarials based on quinoline-triazole scaffold, we have designed hybrid compounds in such a way that [1,2,3]-triazole group is linked to 7-chloroquinoline by an aliphatic diamine linker juxtaposed to a carbonyl group (quinoline-NH-R-NH-carbonyl-triazole, where R is an aliphatic group) (**Scheme 1**). The advantage that hybrid molecules possess, is the ability to surpass the threat of drug resistance due to the presence of multiple pharmacophores and hence multiple mechanism of actions. In 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 synthesis, characterization and antimalarial activity evaluation 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 synthesized via Cu(I) catalyzed Huisgen [2+3] cycloaddition reaction of 2-azido-N-(7-chloroquinolin-4-ylamino-alkyl)acetamide (**4a-d**) and terminal alkynes (**5** and **6a-e**) in $^{t}BuOH:H_2O$ (1:1) mixture containing catalytic amount of copper sulfate and sodium ascorbate at ambient temperature (**Scheme-1**). For making the target molecules, initially alkynes (**6a-e**) were prepared in one step by reacting substituted phenols with propargyl bromide in presence of K_2CO_3 in DMF at room temperature for 8-10 hours.[26,27] The desired azides (**4a-d**) were synthesized in three steps. Firstly, N'-(7-chloroquinolin-4-yl)alkanediamines (**2a-d**) were obtained by heating alkanediamines with 4,7-dichloroquinoline (**1**) at 130^{o}C for 7-8 hours according to the literature procedure.[28,29] In subsequent step, the free amino group of N'-(7-chloroquinolin-4-yl)alkane-diamines (**2a-d**) was coupled with chloroacetyl chloride in DMF at ambient temperature to afford the compounds (**3a-d**) which on heating with sodium azide in methanol at reflux temperature for 7-8 hours afford desired precursors (**4a-d**) in almost quantitative yields. Finally, the synthesis of desired compounds (**7, 8a-b, 9a-e, 10a-b** and **11a-b**) was accomplished in good to excellent yields via Cu(I)-catalysed Huisgen [2+3] cycloaddition reaction 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 established on the basis of spectral analysis and their characterization data are presented in the experimental section.



Scheme 1. Synthesis of 7-chloroquinoline based [1,2,3]-triazole hybrids **7**, **8a-b, 9a-e, 10a-b, 11a-b**: a) 130^{o}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%.

The IR spectrum of a representative compound **9e** has shown four characteristic absorption peaks at 3274, 1686, 1656 and 1599 cm^{-1} corresponding to the stretching frequency of NH, C=O, CHO and C=N. In proton NMR of **9e**, four characteristic peaks were observed at δ 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. The mass spectral analysis gave 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**) by showing a $[M + H]^+$ ion peak at m/z 479 for

the molecular formula $C_{24}H_{23}ClN_6O_3$. The remaining compounds were similarly characterized and their characterization data are presented in the experimental section.

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

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

Selectivity assays

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

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

Since CQ resistance has spread its wings globally[30] especially in *P. falciparum*, and almost all malaria prone areas are gradually becoming CQ resistant, it was imperative to see whether these compounds were showing any signs of cross resistance with CQ since they also possess the same 7-chloroquinoline moiety. Therefore, antimalarial activity of selected compounds namely **8a**, **8b** and **9c** was evaluated against CQ resistant field isolate collected from one of the most malaria endemic areas of India (Rourkela, Odisha). Compounds **8a**, **8b** and **9c** exhibited <100 nM IC_{50} against CQ resistant field isolate (RKL-9) of *P. falciparum* **(Table 1)**. Out of these three compounds, compound **8b** was found to be most potent as evident from its lowest IC_{50} value of 2.94 nM followed by **9c** with IC_{50} of 16.01 nM. Compound **8a** was found least active with IC_{50} value 75.01 nM. The respective dose-response curves are given in Figure S1 (**ESI**).

The activity results revealed that the replacement of halogen moiety (from chlorine to bromine) at *para* position of phenoxy group remarkably increases the potency of the compound against CQ resistant *P. falciparum* by ~25 fold (IC_{50} 2.94 nM vs 75.01 nM). Moreover, an ethyl side chain (instead of propyl side chain) attached to 4-amino-7-chloroquinoline increases antimalarial potency by ~5 folds (IC_{50} 2.94 nM vs 16.01 nM). Although compound **8a**, **8b** and **9c** bear structural similarity with CQ due to the presence of 7-chloroquinoline moiety but the potency data demonstrates that, none of them show any significant signs of cross resistance with CQ thereby demonstrating comparatively higher potency as compared to CQ (IC_{50} (CQ) 114.4 nM) against CQ resistant *P. falciparum*. Collectively, compounds **8a**, **8b** and **9c** demonstrated greater potency as compared to 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 antimalarials.

Antimalarial activity against gametocytes

For carrying out the drug sensitivity experiments, mature gametocytes were produced from the same field isolate (RKL-9) which was previously used for measuring the asexual stage antimalarial activity. The compounds, **8a**, **8b** and **9c** were clearly found to target mature gametocytes and induce morphological deformations in them in a dose dependent manner. Compound **8b** showed maximum potency with IC_{50} (NM) (Normal Morphology) of 8.50 µM and was considered as most active out of three tested compounds. Other tested compounds, **8a** and **9c** also targeted gametocytes with IC_{50} (NM) as 10.71 µM and 12.03 µM, respectively. IC_{50} (NM) and IC_{50} (Total) values for the tested compounds are mentioned in **Table 1**. The respective dose-response curves are given in **Figure S2** (**ESI**). Microscopic images of morphological deformations induced by treatment with these hybrids along with microscopic images of healthy/untreated gametocytes are given in **Figure S3** (**ESI**).

Conclusions

In summary, 4-amino-7-chloroquinoline based [1,2,3]-triazole hybrids were synthesized via a click chemistry approach and were screened for their potential antiplasmodial activity. All the compounds demonstrated promising activity in nanomolar range against CQ sensitive strain (3D7) of *P. falciparum*. Three hybrids (**8a**, **8b** and **9c**) were also found to be active against CQ resistant field isolate of *P. falciparum* (RKL-9) and proved to be more potent than CQ, suggesting minimum possibility of any cross resistance with CQ. Furthermore, these compounds also demonstrated potency against mature gametocytes which is suggestive of their potential transmission blocking activity. These compounds also exhibited no evidence of significant cytotoxicity against mammalian cell line (VERO). Overall, the promising schizonticidal and gametocytocidal activity displayed by the best of the quinoline-triazole hybrids described here encourage further studies on identifying newer hybrids, especially as most quinoline-based schizonticidal drugs are relatively inactive against mature gametocytes.[38] Collectively,

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

Compds.	R	n	Asexual stage	Gametocyte stage			Cytotoxicity	
			3D7	RKL-9	RKL-9		VERO	
			IC_{50}[a]	IC_{50}[a] (95% CI)	IC_{50} NM[b] (95% CI)	IC_{50} Total[b] (95% CI)	CC_{50}[c]	SI[d]
7	-	2	137.10	-	-	-	41.53	304.78
8a	Cl	1	39.98	75.01 (60.53 to 92.96)	10.71 (7.70 to 14.88)	20.87 (17.30 to 25.16)	86.75	2223.72
8b	Br	1	40.00	2.94 (1.96 to 4.43)	8.50 (6.76 to 10.67)	19.80 (15.06 to 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 to 23.65)	12.03 (10.05 to 14.3)	21.91 (17.79 to 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 to 168.4)	-	-	112.44	>20000

[a]IC_{50} values are in nanomolar (nM) concentration. [b]IC_{50} values are in micromolar (μM) concentration. [c]CC_{50} values are in micromolar (μM) concentration. [d]SI (CC_{50}/IC_{50}) of 3D7 strain. CC_{50} = Concentration of test compound inhibiting 50% growth of VERO cells. CI = Confidence Interval. NM = Normal Morphology. SI = Selectivity Index.

these results indicate that the new hybrids with carbonyl group juxtaposed to alkylamino linker connecting 4-amino-7-chloroquinoline base and [1,2,3]-triazole may have the potential to be considered as prototypes for the development of next generation antimalarials.

Experimental Section

Materials and methods

All the chemicals were purchased from Sigma-Aldrich unless otherwise mentioned and used without any further purification. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} pre-coated aluminium plates from Merck and spots were developed either under UV light or in iodine chamber. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO-*d*$_6$ on Jeol ECX-400P (400 MHz) NMR spectrometer. The coupling constants (J) are reported in Hertz (Hz). IR spectra were recorded on a Perkin Elmer IR spectrometer and absorption maxima (υ_{max}) are given in cm^{-1}. Mass spectra were recorded on Waters Micromass LCT ESI-MS spectrometer in positive ionization mode. Elemental analyses were determined on Elementar Analysen systeme GmbH VarioEL V3.00. The melting points were determined in open capillary tubes on Buchi melting point apparatus and are uncorrected. *N*^{1}-(7-chloro-quinolin-4-yl)-alkanediamines (**2a-d**) were prepared according to the literature methods and their characterization data were matched with the reported data.[28,29]

General procedure for the synthesis of compounds 3a-d. To a well stirred solution of *N*^{1}-(7-chloro-quinolin-4-yl)-alkanediamines (**2a-d**; 1.0 mmol) in N,N-dimethylformamide (10 mL), chloroacetyl chloride (4.0 mmol) was added and the reaction mixture was stirred at ambient temperature for 4-5 hours. After the 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 using 10% aqueous NaOH solution. The resulting solution was then extracted with ethyl acetate (60 mL×3 times). The ethyl acetate layer was washed with water (40 mL×3 times) and the organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated under vacuum to furnish the products (**3a-d)** in good yields. These compounds were obtained in a sufficiently pure form and were used as such for further reactions without any purification.

2-Chloro-N-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-acetamide (3a). Dark brown solid; yield: 92%; mp: 160°C; IR (Nujol): υ_{max}=3361 (NH), 3194 (NH), 1649 (C=O), 1584 (C=N), 1455, 1222, 1138, 897, 871, 849, 808, 762, 722 cm^{-1}; ^{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 (m, 4H, $2CH_2$) ppm; MS (ESI) *m/z*: 298 $[M+H]^+$.

2-Chloro-N-[3-(7-chloro-quinolin-4-ylamino)-propyl]-acetamide (3b). Off white solid; yield: 96%; mp: >200°C; IR (Nujol): υ_{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}; ^{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 (m, 2H, CH_2) ppm; ^{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; MS (ESI) *m/z*: 312 $[M+H]^+$.

2-Chloro-N-[4-(7-chloro-quinolin-4-ylamino)-butyl]-acetamide (3c). Dark brown solid; yield: 90%; mp: >200°C; 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}; ^{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 (m, 4H, $2CH_2$) ppm; MS (ESI) *m/z*: 326 $[M+H]^+$.

2-Chloro-N-[6-(7-chloro-quinolin-4-ylamino)-hexyl]-acetamide (3d). Dark brown solid; yield: 78%; mp: >200°C; 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}; ^{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 (m, 6H, $3CH_2$) ppm; MS (ESI) *m/z*: 354 $[M+H]^+$.

General procedure for the synthesis of compounds 4a-d. To a well stirred solution of compound (**3a-d**; 1.0 mmol) in MeOH (60 mL), sodium azide (6.0 mmol) was added and the resulting solution was refluxed for 7-8 hours. After the completion of the reaction, the solvent was evaporated under vacuum to dryness. Thus, the residue obtained was dissolved in ethyl acetate (80 mL). The resulting ethyl acetate solution was washed with water (40 mL×3 times), dried over anhydrous Na_2SO_4 and evaporated under vacuum to furnish the desired products (**4a-d)** in good yields. The products (**4a-d**) were obtained in sufficiently pure form and used as such for next step without any further purification.

2-Azido-N-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-acetamide (4a). Yellow solid; yield: 79%; mp: 154°C; 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}; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.40 (d, *J*=5.13 Hz, 1H, quinoline H-2), 8.35 (brs, 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 (m, 4H, $2CH_2$) ppm; ^{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; 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; 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}; ^{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 (brs, 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, $2CH_2$), 1.84-1.81 (m, 2H, CH_2) ppm; ^{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; MS (ESI) *m/z*: 319 $[M+H]^+$.

2-Azido-N-[4-(7-chloro-quinolin-4-ylamino)-butyl]-acetamide (4c). Off white solid; yield: 91%; mp: 168°C; 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}; ^{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_1=8.79 Hz, J_2=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 (m, 2H, CH_2) ppm; ^{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; MS (ESI) *m/z*: 333 $[M+H]^+$.

2-Azido-N-[6-(7-chloro-quinolin-4-ylamino)-hexyl]-acetamide (4d). Dark brown solid; yield: 88%; mp: 124°C; 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}; ^{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_1=8.79 Hz, J_2=2.20 Hz, 1H, quinoline H-6), 7.36 (brs, 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 (m, 6H, $3CH_2$) ppm; ^{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; MS (ESI) *m/z*: 361 $[M+H]^+$.

General procedure for the synthesis of compounds 7, 8a-b, 9a-e, 10a-b and 11a-b. To a solution of azide (**4a-d**; 3.0 mmol) in 50% *t*-butanol in water (20 mL), alkyne (**5** or **6a-e**; 3.5 mmol), $CuSO_4.5H_2O$ (1.0 mmol) and sodium ascorbate (2.2 mmol) were added. The reaction mixture was stirred at room temperature for 8 hours. After the completion of 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 times). The ethyl acetate layers were combined, dried over anhydrous Na_2SO_4 and evaporated under vacuum. Thus, the crude product obtained was stirred in diethyl ether (60 mL) at room temperature for 2 hours. The solid was then filtered, washed well with Et_2O and dried under vacuum to obtain the pure product in good to excellent yields.

N-(3-(7-Chloroquinolin-4-ylamino)propyl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetamide (7). Pale yellow solid; yield: 87%; mp: >200°C; IR (Nujol): ν_{max}=3273 (NH), 1654 (C=O), 1583 (C=N), 1455, 1377, 1079, 976, 846, 805, 762, 721, 689 cm^{-1}; ^{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, $2CH_2$), 1.86-1.80 (m, 2H, CH_2); MS (ESI) *m/z*: 421 $[M + H]^+$.

2-[4-(4-Chloro-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-acetamide (8a). White solid; yield: 85%; mp: 190°C; 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}; ^{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 (m, 4H, 2 CH_2) ppm; ^{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; MS (ESI) *m/z*: 471 $[M + H]^+$. Anal. calcd for $C_{22}H_{20}Cl_2N_6O_2.0.2H_2O$: C, 55.64; H, 4.33; N, 17.69, found: C, 55.57; H, 4.45; N, 17.57.

2-[4-(4-Bromo-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-acetamide (8b). Dirty white solid; yield: 87%; mp: 184°C; IR (Nujol): ν_{max}=3387 (NH), 3273 (NH), 1654 (C=O), 1582 (C=N), 1458, 1376, 1237, 1172, 1139, 1054, 811, 721 cm^{-1}; ^{1}H

NMR (400 MHz, [D6]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 (m, 4H, $2CH_2$) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 516 $[M+H]^+$. Anal. calcd for $C_{22}H_{20}ClBrN_6O_2.0.6\ H_2O$: C, 50.18; H, 4.06; N, 15.96, found: C, 50.18; H, 3.92; N, 15.64.

N-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-2-(4-phenoxymethyl-[1,2,3]triazol-1-yl)-acetamide (9a). Pale yellow solid; yield: 78%; mp: 174°C; 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}; ^1H NMR (400 MHz, [D6]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 (m, 2H, CH_2) ppm; MS (ESI) *m/z*: 451 $[M+H]^+$. Anal. calcd for $C_{23}H_{23}ClN_6O_2.0.8\ H_2O.0.1C_4H_{10}O$: C, 59.45; H, 5.46; N, 17.78, found: C, 59.41; H, 5.54; N, 17.44.

2-[4-(4-Chloro-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[3-(7-chloro-quinolin-4-ylamino)-propyl]-acetamide (9b). Pale yellow solid; yield: 82%; mp: 192°C; 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}; ^1H NMR (400 MHz, [D6]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 (m, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 486 $[M+H]^+$. Anal. calcd for $C_{23}H_{22}Cl_2N_6O_2.0.89\ H_2O$: C, 55.10; H, 4.78; N, 16.76, found: C, 54.93; H, 4.45; N, 16.42.

2-[4-(4-Bromo-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[3-(7-chloro-quinolin-4-ylamino)-propyl]-acetamide (9c). Pale yellow solid; yield: 77%; mp: 202°C; 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}; ^1H NMR (400 MHz, [D6]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_1=6.59 Hz, J_2=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_1=7.69 Hz, J_2=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 (m, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 530 $[M+H]^+$. Anal. calcd for $C_{23}H_{22}ClBrN_6O_2.0.3\ H_2O$: C, 51.61; H, 4.26; N, 15.70, found: C, 51.73; H, 4.53; N, 15.38.

N-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-2-(4-p-tolyloxymethyl-[1,2,3]triazol-1-yl)-acetamide (9d). Light brown solid; yield: 79%; mp: 196°C; 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}; ^1H NMR (400 MHz, [D6]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, $2CH_2$), 2.23 (s, 3H, CH_3), 1.86-1.83 (m, 2H, CH_2) ppm; MS (ESI) *m/z*: 465 $[M+H]^+$. Anal. calcd for $C_{24}H_{25}ClN_6O_2.H_2O$: 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)-1H-1,2,3-triazol-1-yl)acetamide (9e). Pale yellow solid; yield: 88%; mp: 182°C; 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}; ^1H NMR (400 MHz, [D6]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, $2CH_2$), 1.86-1.81 (m, 2H, CH_2) ppm; MS (ESI) *m/z*: 479 $[M+H]^+$.

2-[4-(4-Chloro-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[4-(7-chloro-quinolin-4-ylamino)-butyl]-acetamide (10a). Pale yellow solid; yield: 76%; mp: 198°C; 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}; ^1H NMR (400 MHz, [D6]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 (m, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 500 $[M+H]^+$. Anal. calcd for $C_{24}H_{24}Cl_2N_6O_2.1.4\ H_2O$: C, 54.95; H, 5.15; N, 16.02, found: C, 55.15; H, 4.93; N, 15.63.

N-[4-(7-Chloro-quinolin-4-ylamino)-butyl]-2-(4-p-tolyloxymethyl-[1,2,3]triazol-1-yl)-acetamide (10b). White solid; yield: 82%; mp: 202°C; 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}; ^1H NMR (400 MHz, [D6]DMSO): δ=8.37 (brs, 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 (m, 4H, CH_2) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 479 $[M+H]^+$.

2-[4-(4-Chloro-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[6-(7-chloro-quinolin-4-ylamino)-hexyl]-acetamide (11a). White solid; yield: 79%; mp: 116°C; 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}; ^1H NMR (400 MHz, [D6]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_1=9.15 Hz, J_2=2.20 Hz, 1H, quinoline H-6), 7.32-7.29 (m, 3H, ArH and NH), 7.04 (dd, J_1=7.32 Hz, J_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 (m, 6H, $3CH_2$) ppm; ^{13}C NMR (100 MHz, [D6]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; MS (ESI) *m/z*: 528 $[M+H]^+$. Anal. calcd for $C_{26}H_{28}Cl_2N_6O_2.0.7\ H_2O$: C, 57.82; H, 5.49; N, 15.56, found: C, 57.82; H, 5.56; N, 15.27.

2-[4-(4-Bromo-phenoxymethyl)-[1,2,3]triazol-1-yl]-N-[6-(7-chloro-quinolin-4-ylamino)-hexyl]-acetamide (11b). White solid; yield: 82%; mp: 186°C; 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}; ^1H 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 (m, 8H, $4CH_2$) ppm; ^{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; MS (ESI) *m/z*: 572 $[M+H]^+$.

Biological assays

Culture adaptation and in vitro cultivation of asexual stages. *P. falciparum* cryo-preserved strains (CQ sensitive-3D7 and CQ resistant-RKL9, a field isolate collected from Rourkela, India) were revived according to standard protocols and introduced in culture with the objective of 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 (GIBCO) (with glutamine) containing 25 mM HEPES, 2g/L D-glucose, 2 g/L sodium bicarbonate, 40 µg/ml gentamicin sulfate 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 at 37ºC in a CO_2 incubator in the presence of 5% CO_2. Synchronous parasites at ring stages were obtained by treatment with 5% sorbitol (w/v) as per requirement.

In vitro production of gametocytes: The detailed procedures for gametocyte production is described elsewhere.[33] Briefly, on the day of setting up the gametocyte culture (day 0), asexual culture was sorbitol-synchronized to get >90% ring stages and parasitaemia was lowered to 0.5% by addition of fresh erythrocytes (10% haematocrit). These parasites were daily replenished with complete RPMI-1640 media supplemented with hypoxanthine (50 µg/mL) and kept devoid of fresh erythrocytes throughout the period of culture maintenance. Haematocrit was reduced to 5% on day 8 and 50 mM N-Acetyl-Glucosamine (NAG) 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 sensitivity of compounds.

Selection of most potent compounds using Malaria SYBR-green-I based Fluorescent (MSF) Assay: All twelve quinoline-triazole hybrids were initially screened for their antimalarial activity using Malaria SYBR green-I based Fluorescence assay[34] to select most potent compounds for a detailed investigation. Appropriate stock solutions of compounds and standard drug CQ were prepared in DMSO (final DMSO concentrations <0.5% v/v) and water respectively and stored at -20ºC until use. Appropriate working solutions were made afresh on the day of the experiment with complete culture medium. Two fold serial dilutions of test samples were prepared in triplicates in 96 well microtiter plates and incubated with 1% parasitized red blood cell suspension having 0.8-1% parasitaemia (>90% rings, sorbitol synchronized). Positive control and negative control wells were also prepared without the test compounds, with 1% parasitized and non-parasitized erythrocyte suspension, respectively. Unused parasite culture was kept separately without any drug for monitoring the parasite growth. Plates were incubated at 37ºC for 72 hours in presence of 5% CO_2 and air mixture. After incubation period, 100 µl of lysis buffer (Tris, EDTA, Saponin, Triton X-100, 2x concentration of SYBR-Green I (Invitrogen) in water) was added to each well and incubated in dark for one hour at 37ºC. The plates were examined at 485±20 nm of excitation and 530±20 nm of emission for Relative Fluorescence Units (RFUs) per well using the fluorescence plate reader. The half maximal inhibitory concentration values (IC_{50}) for each compound were determined using nonlinear regression analysis of dose-response curves generated using pre-programmed excel spread sheet after two independent experiments.

Evaluation of antimalarial activity against chloroquine resistant *P. falciparum*: Selected compounds which showed promising activity against 3D7 were further tested for their antimalarial potential against CQ resistant field isolate (RKL-9) of *P. falciparum* using a Schizont Maturation Inhibition assay (SMI).[35-37] Briefly, for carrying out dose response evaluation, two fold dilutions of test compounds in desired concentrations were prepared in 96 well microtiter plates. Inhibition experiments were typically carried out at a final concentration of 4 µM. Sorbitol-synchronized parasites with 1% parasitaemia (>90% rings) were added in each well during the start of experiment. 100 nM DHA (Dihydroartemisinin) and 0.5% DMSO (v/v) were used as positive and negative (vehicle) control, respectively. Separate wells without any test compounds/drugs were used to monitor uninhibited growth. Microtiter plates were incubated at 37ºC in the presence of 5% CO_2 for 22-36 hours, till >10% schizont maturation (schizonts with 4 or more merozoites) in untreated wells is observed. After the end of incubation period, thin smears were prepared from each well and stained with 10% Giemsa for 20 minutes. 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 log of concentration using a non-linear 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 triplicates in three separate occasions.

Gametocytocidal assays and data analysis: Gametocytocidal drug assays were carried out in three independent experiments and potency evaluation performed as described elsewhere.[33] Briefly, gametocytes with majority of late stages were harvested on the day of experiment and thorough systematic morphological examination was performed by light microscopy before carrying out the screening experiments. Test plates were prepared by plating two fold dilutions of the drugs in duplicates to achieve the concentrations upto 50 µM for test compounds and 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 10 µM methylene blue were used as vehicle (negative) and positive controls, respectively. Plates were incubated at 37°C for 48 hours in presence of 5% CO_2.[38] After incubation period, thin smears were prepared, stained with 10% Giemsa and examined under a 100x oil immersion objective.[39] Five thousand erythrocytes from each smear were counted to examine the gametocytaemia and gametocyte morphology at each concentration. Late stage gametocytes (Stage IV and V) observed were morphologically categorized into two groups, 1) Normal Morphology (NM) group containing healthy gametocytes and 2) Altered Morphology (AM) group containing morphologically deformed/unhealthy gametocytes. Gametocytaemia for each concentration was expressed as percentage inhibition compared to drug-free control which was plotted against log of concentration using a non-linear regression analysis (four parameter log dose with variable slope) to compute IC_{50} values and 95% confidence intervals. Dose-response curves expressed as percentage inhibition vs. 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 labelled as 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 studies were carried out against VERO cells (C 1008; Monkey Kidney Fibroblast) using MTT cell viability assay by following the published protocols with minor modifications.[40]

Briefly, 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 at 37°C in the presence of 5% CO_2 for 72 hours. MTT (5mg/ml) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was added to each well and plates were incubated in the dark for 4 hours. The by-product (dark blue formazan) formed by viable cells was dissolved in DMSO and absorbance was recorded at 550 nm using ELISA reader. 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis. Selectivity indices (SI) were calculated for each compound using IC_{50} values obtained for 3D7 strain of *P. falciparum*, as follows:

$$SI = {CC50}/{IC50}$$

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: 7-chloroquinolines • [1,2,3]-triazole hybrids • Plasmodium falciparum • antimalarial activity • dual-stage antimalarials • gametocyte • synthesis

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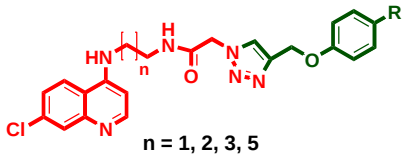

n = 1, 2, 3, 5
R = H, Cl, Br, CH_3, CHO
(IC_{50} = 38.75 - 910.14 nM)

Dual stage antimalarials: Novel hybrids based on 4-amino-7-chloroquinoline and [1,2,3]-triazole scaffolds were synthesized and evaluated for their antiplasmodial activity against asexual blood stages and gametocytes of *P. falciparum*. Majority of the compounds displayed <100 nM IC_{50} values against CQ sensitive strain (3D7) and exhibited no significant signs of cytotoxicity against mammalian cell line (VERO). Three best compounds were appreciably more active (IC_{50} values <100 nM) than chloroquine against CQ resistant field isolate RKL-9 and also demonstrated potency against mature gametocytes.