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ORIGINAL RESEARCH

Synthesis, characterization and antimalarial activity of isoquinoline derivatives

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Abstract

This paper presents synthesis of novel decorated isoquinolines that can be used as antimalarial agents. Two series of compounds, isoquinoline phenyl and isoquinoline triazole derivatives, were synthesized via the Suzuki–Miyaura and Sharpless–Fokin reactions respectively. The final compounds were investigated for their antimalarial activity in cell-based experiments. In the series of isoquinoline phenyl derivatives, compound **6** exhibited interesting antiplasmodial activity against chloroquine resistant (K1) and chloroquine sensitive (3D7) strains of *P. Falciparum* with IC_{50} 1.91 ± 0.21 μM and 2.31 ± 0.33 μM, respectively. In the series of isoquinoline-triazole derivatives, compound **15** was the most active against resistant (K1) and sensitive (3D7) strains of *P. falciparum* with IC_{50} 4.55 ± 0.10 μM and 36.91 ± 2.83 μM, respectively. The respective resistance indices of compounds **6** and **15** against K1 and 3D7 were 0.83 and 0.12. Compound **15** was promisingly effective against the resistant strain. The results of this study provided useful contributions for further lead discovery and lead modification of modern rational drug design.

Keywords Antimalarial activity · *Plasmodium falciparum* · Chloroquine resistant · Isoquinoline · 1,2,3-triazole

Abbreviations

P.	Plasmodium
Pfcrt	Plasmodium falciparum chloroquine resistant transporter
Pfmdr1	Plasmodium falciparum multidrug resistance 1

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Introduction

Malaria is a major disease and public health concern. It is mainly prevalent in Africa, South-East Asia and the Eastern Mediterranean. Malaria is caused by *Plasmodium* parasite. The main transmission vector is the female Anopheles mosquito [1, 2]. Mosquitoes taking a blood meal on infected individuals ingest parasites into the midgut. Subsequently, the parasites move into the mosquito's salivary glands and subsequently infect a human host [3–6]. Humans can be infected with one or more of the following five strains: *P. falciparum, P. vivax, P. malariae*, *P. ovale* and *P. knowlesi* [4–7]. Currently, *P. falciparum* and *P. vivax* are the prevalent protozoan species causing high mortality, *P. falciparum* being the particular most virulent species [2]. The clinical symptoms of malaria are mainly caused by schizont rupture and destruction of erythrocytes. Major symptoms of malaria are fever, chills, headaches, and diaphoresis [8].

Currently, the commercially available antimalarial drugs are quinoline derivatives, artemisinin derivatives, antifolates and some antimicrobial agents [9]. Antimalarial agents principally aim to inhibit one or two stages in the life cycle of the parasite. Among these drugs, quinine and chloroquine

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are the oldest drugs and the first choice for treating all species of malaria [10]. An artemisinin-based combination therapy based regimen could be applied where chloroquine is ineffective in eradiacating malaria [10]. However, the emergence of drug resistance to available antimalarial drugs is a serious problem [11]. Two of the five human malaria parasite species, *P. falciparum* and *P. Vivax*, are frequently resistant. The incidence of chloroquine resistance of *P. falciparum* is a major problem for malarial control. Both artemisinin and its partner drugs are also threatened, especially in Africa [2]. On the other hand, *P. malariae* or *P. ovale* remain unknown for current drug resistance [4–6]. Genetic mutations or genetic malfunction on the major pathways of drug resistance, i.e., enzymes or transporters, are the main factors leading to drug resistance [12]. The examples of multidrug resistant strains of *P. falciparum* are Dd2, K1 and W2 [13]. The most common cause of chloroquine resistance is derived from mutation in the Pfmdr1 and Pfcrt genes in *P. falciparum* [14, 15].

To afford better malaria control, many researchers have sought new compounds or to modify the molecular structure of available current drugs such as chloroquine [10, 16]. The mechanism of chloroquine is inhibition of hemozoin formation. Normally, the parasites digest hemoglobin to release free heme which is toxic to the parasites themselves. However, parasites have a self-protective mechanism, crystallizing heme to hemozoin which is insoluble and harmless for the parasites [17, 18]. Chloroquine is able to prevent the formation of hemozoin. In particular, the current proposed mechanism is accumulation of chloroquine in acidic digestive vacuoles of intraerythrocytic trophozoites by a weak base mechanism [19]. At physiological pH, chloroquine can exist in three forms: unprotonated, monoprotonated, and diprotonated forms. Only uncharged chloroquine can permeate the erythrocyte membrane and finally reach the vacuole. In this vacuole, displaying acid condition chloroquine molecules become protonated and, since membranes are not permeable to charged species, the drug accumulates into the acidic digestive vacuole [20, 21]. Another mechanism of chloroquine, the inhibition of heme polymerase in the food vacuole of the malaria trophozoite, results in a lack of essential amino acids and leads to parasite death. There have been several studies on synthesis of chloroquine derivatives including their metal complexes to optimize the bioactivity of synthesized compounds [10]. The substituted chloroquine derivatives were reported as having interesting antimalarial activity. Azoles are an important class of heterocyclic compounds containing nitrogen with various biological activities [22, 23]. The linker of azoles with the core quinoline structure was known to increase its antimalarial activities [10]. A plausible reason for this was heme complexation through azoles and presented antimalarial activity [23].

The aim of this study is to find new compounds with antimalarial properties, particularly the ability to tackle the chloroquine resistant Plasmodium strains. Naturally, we maintained the core structures which preserve nitrogen atoms in the ring, similar to quinoline, but we decided to alter the position of nitrogen atoms probing the antimalarial activity of isoquinolines and attempt to synthesize quinolines containing a phenyl or triazole ring as shown in Fig. 1. We decorated the isoquinoline scaffold with two reactions, namely (i) the Suzuki–Miyaura reaction and (ii) the Sharpless–Fokin reaction to afford two series; isoquinoline-phenyl and isoquinoline-triazole (Fig. 1).

The modification of the two series is displayed in Fig. 2. All synthesized compounds were programmed to examine biological activity in two strains, the chloroquine-sensitive strain (3D7) and the chloroquine resistant strain (K1) *Plasmodium falciparum*. We determined the resistance index (Ri) as the ratio of the IC_{50} value of the resistant strain to that of the sensitive strain. The resistance indices of K1 and 3D7 were calculated and eventually assessed for any relationship between structures and activities of compounds.

Results and discussion

Chemical synthesis

To generate new compounds, we rationalized the structure of the isoquinoline core structure and modified it in two series (Fig. 2), isoquinoline phenyl derivatives **1–13** and isoquinoline triazole derivatives **14–32**. Isoquinoline phenyl derivatives were prepared using the Suzuki–Miyaura reaction (Scheme 1). This cross-coupling reactions were in which commercially available 4-bromoisoquinoline and different aryl boronic acid in the presence of palladium as a catalyst

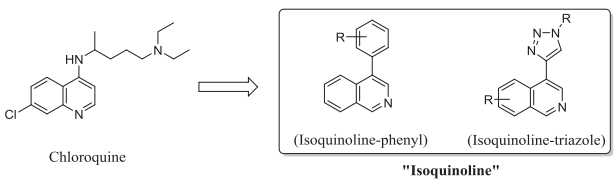


Fig. 1 Concept of structural modification

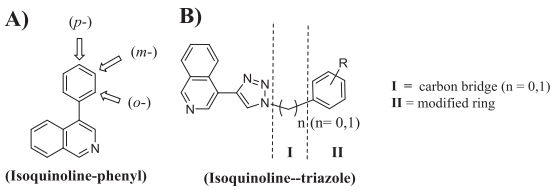
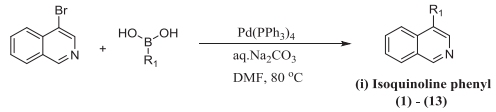
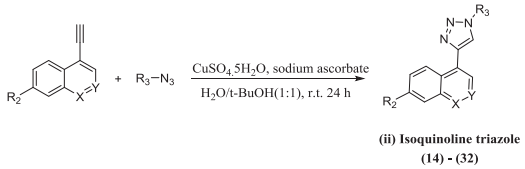


Fig. 2 Modified structures of the two drug series **a** isoquinoline-phenyl and **b** isoquinoline-triazole



Scheme 1 Synthesis of compounds **1–13** via the Suzuki–Miyaura reaction



Scheme 2 Synthesis of compounds **14–32** via the click reaction

under basic conditions. For isoquinoline triazole derivatives, we proceeded to synthesize by using the click reaction (Scheme 2). The archetypical click chemistry is a two-component reaction in which an alkyne and an azide react in the presence of copper(I) catalyst to give rise to a 1,4-disubstituted 1,2,3-triazole. We therefore use different azide compounds bearing the desired triazoles. The azide compounds were prepared as described in Utsintong et al. [24].

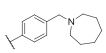
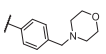
The average percentage yields of isoquinoline phenyl derivatives, compounds **1–13** were more than 70 (Table 1). Compound **2** obtained the lowest percentage yield (20%) possibly due to the presence of an unprotected hydroxyl group. In the series of isoquinoline triazole derivatives, compounds **14–32**, the 1,2,3-triazole ring was easily obtained via azide-alkyne cycloaddition in the presence of a small catalytic amount of copper (I) giving the desired products with percentage yields ranging from 19 to 88% (Table 2). All synthesized compounds were purified by column chromatography and characterization by FTIR and ^{1}H-NMR. Full characterization was performed on the compounds most active against *P. falciparum*. The typical aromatic ring band at 3100–3000, 1600–1450 cm^{-1} in IR and signal at around δ9.20–8.00 ppm in ^{1}H NMR spectra of all derivatives confirm the presence of isoquinoline ring. The presence of traizole ring in compounds **14–32** was confirmed by the observed 1H singlet at δ 8.3 ppm in ^{1}H NMR spectra and the signal at around δ 133 ppm in ^{13}C NMR. Compound **6** conatins m-OCH_3 and is confirmed by signal at δ 3.85 ppm (3H, singlet) in ^{1}H NMR along with signals at δ 55.3 ppm in ^{13}C NMR. In the mass spectrum of compound **6** $[M+H]^+$ peak was observed confirming the corresponding structure. The complete spectral data along with the assignments are provided in the Experimental section and partial spectra data are provided in the Supplementary section.

Antimalarial activity of synthetic compounds

The IC_{50} values against *P. falciparum* of individual synthesized compounds and four standard antimalarial drugs are summarized in Tables 1–3. All synthesized compounds were active against the 3D7 strain of *P. falciparum*, with IC_{50} from 2.31 to 364.93 µM. The activity of all synthesized compounds against the K1 strain of *P. falciparum* with IC_{50} values from 1.91 to 1104.51 µM. Compound **6** was the most

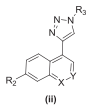
Table 1 Percentage yield and antimalarial activity of isoquinoline phenyl derivatives against chloroquine sensitive (3D7) and chloroquine resistant (K1) strains of *P. falciparum* and resistance index (Ri)

(i)

Entry	R_1	Yield (%)	Antimalarial activity		Resistance index (Ri)
			3D7 IC_{50} (µM)	K1 IC_{50} (µM)	
1		81	26.05 ± 1.99	52.44 ± 6.95	2.01
2		20	27.11 ± 2.24	37.38 ± 1.40	1.38
3		92	30.97 ± 2.40	32.49 ± 1.49	1.05
4		84	47.77 ± 5.35	30.47 ± 2.86	0.64
5		91	30.01 ± 3.61	22.33 ± 2.27	0.74
6		73	2.31 ± 0.33	1.91 ± 0.21	0.83
7		79	42.01 ± 6.99	19.78 ± 3.41	0.47
8		63	203.73 ± 31.41	223.29 ± 25.70	1.10
9		36	29.98 ± 4.10	43.58 ± 3.92	1.45
10		78	73.55 ± 7.63	47.31 ± 3.33	0.64
11		33	35.59 ± 0.82	39.52 ± 7.75	1.11
12		78	41.60 ± 3.93	14.40 ± 0.61	0.35
13		25	21.86 ± 2.14	19.53 ± 3.57	0.89

Ri (resistance index) is the ratio of the IC_{50} of the K1 strain to the IC_{50} of 3D7 strain

Table 2 Percentage yield and antimalarial activity of isoquinoline triazole derivatives against 3D7 and K1 strains of *P. falciparum* and resistance index (Ri)



(ii)

Entry	X	Y	R_2	R_3	Yield (%)	Antimalarial activity		Resistance index (Ri)
						3D7 IC_{50} (μM)	K1 IC_{50} (μM)	
14	C	N	H		35	57.96 ± 11.39	39.11 ± 1.71	0.67
15	C	N	H		42	36.91 ± 2.83	4.55 ± 0.10	0.12
16	C	N	H		73	364.93 ± 16.79	95.76 ± 15.57	0.26
17	C	N	H		43	122.81 ± 4.27	83.18 ± 6.50	0.68
18	C	N	H		25	62.02 ± 5.05	47.72 ± 3.67	0.77
19	C	N	H		88	∝	∝	NA
20	C	N	H		19	139.98 ± 13.91	205.98 ± 17.13	1.47
21	C	N	H		41	57.15 ± 5.05	58.10 ± 5.95	1.02
22	C	N	H		38	12.51 ± 2.02	14.28 ± 2.97	1.14
23	C	N	H		74	35.81 ± 1.76	51.95 ± 5.85	1.45
24	C	N	H		23	34.53 ± 2.96	1104.51 ± 133.17	31.99
25	C	N	H		36	47.46 ± 0.82	11.19 ± 1.72	0.24
26	C	N	H		32	181.36 ± 23.08	91.65 ± 7.78	0.51
27	C	C	H		33	36.97 ± 45.90	42.19 ± 40.46	1.14
28	C	C	H		83	160.25 ± 10.85	65.12 ± 6.93	0.41
29	C	C	H		30	83.87 ± 18.05	91.69 ± 34.70	1.09
30	N	C	Cl		39	130.77 ± 17.24	154.98 ± 14.13	1.19
31	N	C	Cl		39	∝	∝	NA
32	N	C	Cl		32	91.79 ± 6.66	72.05 ± 7.55	0.78

Values are mean ± SDs of three times repetition

NA Not applicable, ∝ excessively high values/undeterminable

active of all 32 compounds with an IC_{50} value of 2.31 ± 0.33 μM and 1.91 ± 0.21 μM against 3D7 and K1, respectively. The less potent compounds were compound **16** with an IC_{50} value of 364.93 ± 16.79 μM against 3D7 and compound **24** with an IC_{50} value of 1104.51 ± 133.17 μM against K1.

Fidock and Kalra categorized antimalarial activity into three classes: potent activity if the IC_{50} is <1 μM, moderately potent if the IC_{50} is between 1 and 5 μM, and weak activity if the IC_{50} is >5 μM [25, 26]. Under this classification, only compound **6** was a moderate antimalarial agent against both strains, while compound **15** was as moderate antimalarial agent against the resistant strain.

In addition, the resistance indices (Ri) of K1 and 3D7 were calculated by finding the ratio between the IC_{50} of the resistant strain (K1) to that of the sensitive strain (3D7). A high index value represents the resistance of the tested compound in the sensitive strain. One-third of the thirty-two compounds were equipotent against both 3D7 and K1 strains. Compounds **12**, **15, 16** and **25** displayed lower Ri values than reference standards (compound **12**: Ri 0.35, **15**: Ri 0.12, **16**: Ri 0.26, **25**: Ri 0.24) (Tables 1–3). Compound **15** was the most potent against K1 strains, with an IC_{50} of 4.55 ± 0.1 μM (Fig. 3).

Regarding the isoquinoline-phenyl series, the results displayed that the position of substituted groups on the phenyl ring connected to the isoquinoline was very important. Compared with compound **1**, substitution with one donor group ($–OCH_3$) in the *meta* position on phenyl ring as compound **6** resulted in a 10 and 25-fold increment in activity against *P.falciparum* 3D7 and K1 respectively. In contrast, the potency of analogues substituted with donor groups ($–OCH_3$, –OH, –SH) or a withdrawing group ($–SO_2CH_3$) in the *ortho/para* position displayed a distinct reduction of activity. This substituition showed improper orientation resulting in activity decrement. In addition, disubstition of methoxy groups at *meta* orientation resulted in a 10–20-fold decrement in antiplasmodial activity. This study demonstrated the possible potential of modification on the phenyl ring in increasing antimalarial activity.

When considering the isoquinoline-triazole series without a methylene bridge between the 1,2,3-triazole and the phenyl ring, the change of substituted position from *para*- to *meta*- on the phenyl ring had a significant effect on antiplasmodial activity against both 3D7 and K1 strains as shown in the resistant index values (Table 2). The results show that the *para*-substituted chlorine in compound **15** was the preferred group on phenyl ring but *meta*-substitution in compound **16** resulted in a tenfold decrement in activity against both 3D7 and K1 strains. Furthermore, the isoquinoline-triazole series with one methylene bridge and substitution at *p*-position of the

Table 3 Antimalarial activity of four current drugs against 3D7 and K1 strains of *P. falciparum* and resistance index (Ri)

Compound	Antimalarial activity		Resistance index (Ri)
	3D7 IC_{50} (μM)	K1 IC_{50} (deM)	
Chloroquine (CQ)	0.007 ± 0.001	0.161 ± 0.014	23
Quinine (Q)	0.098 ± 0.015	0.303 ± 0.013	3.09
Mefloquine (MQ)	0.029 ± 0.003	0.012 ± 0.002	0.41
Dihydroartemisinin (DHA)	0.00456 ± 0.0012	0.00216 ± 0.0005	0.47

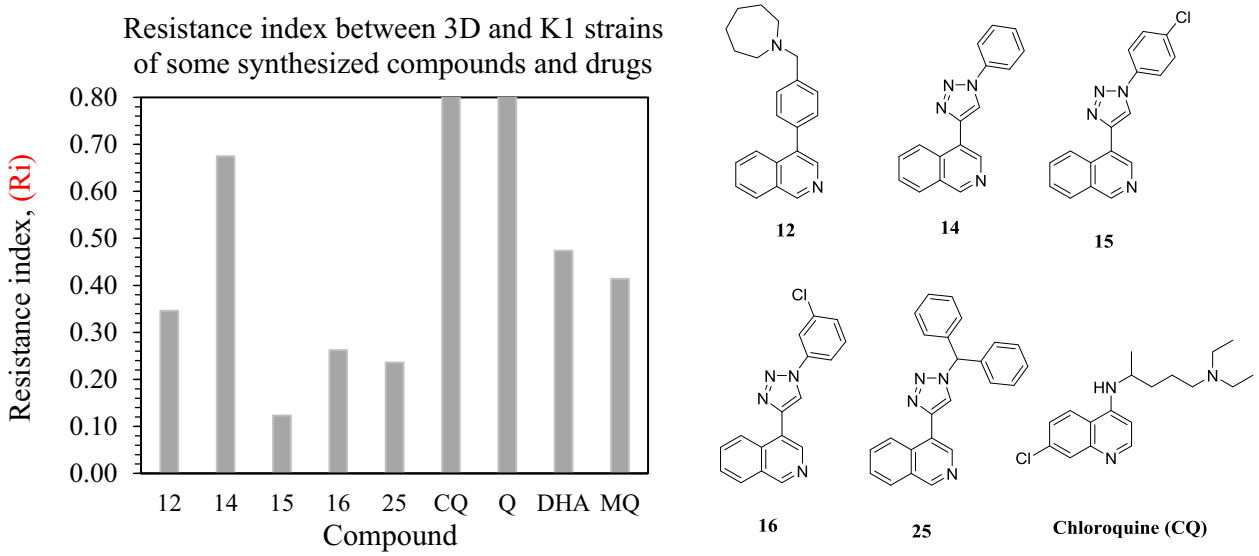


Fig. 3 Resistance index and chemical structures of some synthesized compounds

phenyl ring still maintained activity. Compound **22** with t*ert*-butyl was the most active for antimalarial activity on both strains.

Finally, to understand the importance of the isoquioline ring, this ring was replaced with a naphthyl group. Compounds **27**, **28** and **29** were compared in activity to similar compounds (**22**, **15** and **25**). All modified derivatives demonstrated obviously decrease antimalarial activity in both strains. Furthermore, we attempted to mimic CQ structure by replacing the isoquinoline ring with a 7-chloroquinoline ring as presented in compound **30, 31** and **32**. The results exhibited the effect of the 7-chloroquinoline ring in decreasing their corresponding activity in both strains.

Parasite growth and morphological change cells following exposure to compounds

The morphology change of 3D7 and K1 *P. falciparum* following exposure to selected compounds (compounds **6** and **15**) at IC_{90} were determined. CQ and DHA were used as a positive control and evaluated at IC_{100}. The experiment with no compound exposure was determined as a negative control. The effect of compounds on parasite growth was observed during a 60–72 h incubation period (Fig. 4). Compound **6** inhibited parasite growth and multiplication in both 3D7 and K1 strains since h12 at the ring stage. On the other hand, under negative control, parasites still developed to the trophozoite stage. Parasite shrinkage and death were observed after 12 h incubation. Compound **15** had markedly different antimalarial activity against 3D7 and K1 strains with higher specific activity against K1 than 3D7. In addition, compound **15** was able to delay parasite growth since h24, with early schizonts observed in the control sample, whereas the compound-exposed parasite remained at the trophozoite stage and gradually developed to schizonts at h60 and h72 in K1 and 3D7 respectively. Furthermore, compound **15** may have affected hemozoin formation because abnormal hemozoin crystals were found since h36 (Fig. 4, shown with arrows).

The results displayed the effects of parasite exposure to compounds **6** and **15** for 60 h. Both compounds **6** and **15** could inhibit *Plasmodium falciparum* time-dependent growth compared to the negative control. However, compound **15** was less potent than CQ and DHA. Furthermore, compound **6** and CQ completely inhibited growth of the 3D7 strain while DHA completely inhibited parasite growth

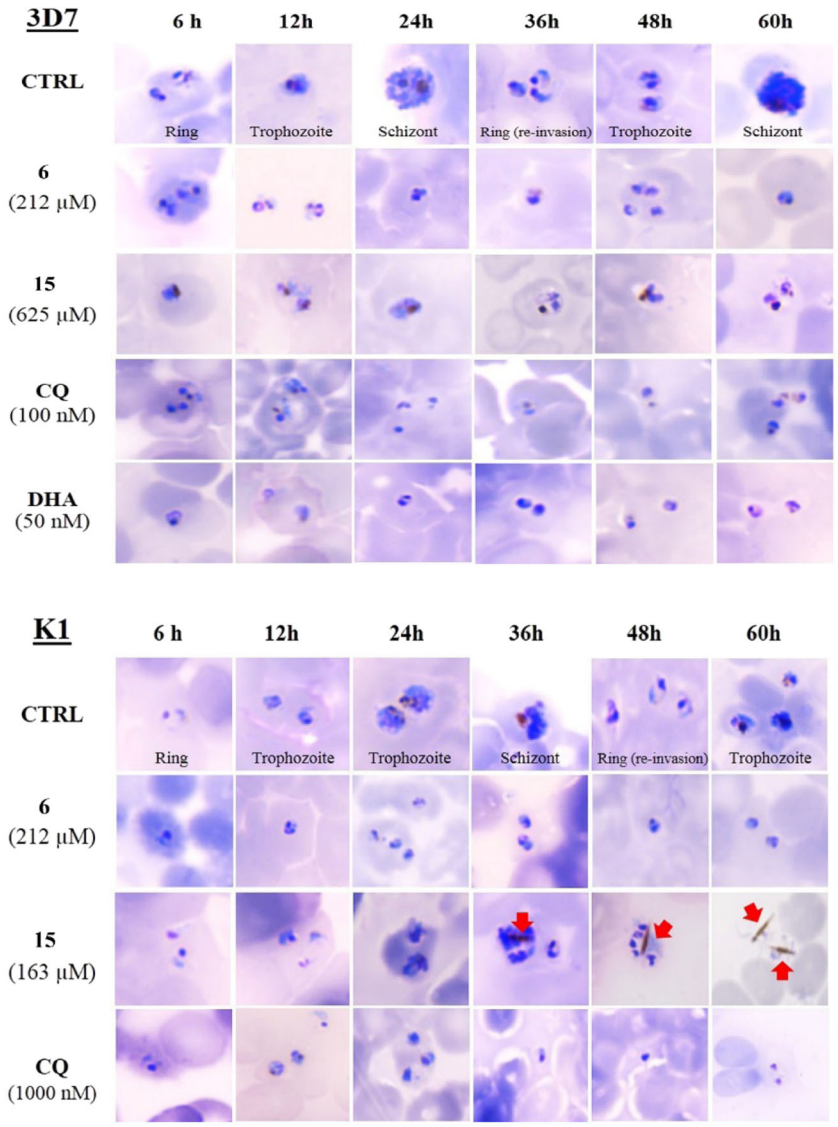
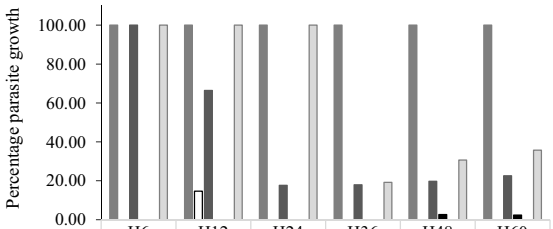


Fig. 4 Morphology of *Plasmodium falciparum* after compound exposure **6**. Two standard antimalarials, chloroquine (CQ) and dihydroartemisinin (DHA), were used as the positive control. Red arrows pointed to abnormal hemozoin crystals found in K1 after 36 h exposure



	H6	H12	H24	H36	H48	H60
3D7 or K1- CTRL	100.00	100.00	100.00	100.00	100.00	100.00
3D7-DHA, 50 nM	0.00	14.67	0.00	0.00	0.00	0.00
3D7-15, 652 uM	100.00	66.45	17.68	17.95	19.75	22.61
K1-CQ, 1000 nM	0.00	0.00	0.00	0.00	2.64	2.40
K1-6, 212 uM	0.00	0.00	0.00	0.00	0.15	0.00
K1-15, 163 uM	100.00	100.00	100.00	19.19	30.69	35.75

Fig. 5 Percentage growth of *Plasmodium falciparum* after exposure to compound **6** at 212 µM concentration and **15** at 163 µM concentration for 60 h. Two standard antimalarial drugs, chloroquine (CQ) at 100 µM concentration and dihydroartemisinin (DHA) at 50 nM concentration were used as a positive control. The vehicle was used as a negative control (CTRL)

in the K1 strain at 6 h of testing exposure (data not presented in chart) (Fig. 5).

Conclusion

In this study, the antimalarial activity of novel synthesized isoquinoline derivatives was evaluated. Compound **6** exhibited antiplasmodial activity compared to reference standard antimalarial drugs. Although compound **6** only displayed moderate antimalarial activity, it was active against both chloroquine sensitive and chloroquine resistant strains. In addition, compound **15** showed interesting selective activity against resistant strains over sensitive strains. Overall this work provides knowledge for further study about the discovery of antimalarial agents, which selectively work on resistant strains.

Experimental section

Chemistry

Unless otherwise noted, all reagents, chemicals and catalysts were purchased from commercial sources (Sigma-Aldrich; USA, Fluorochem; UK). Some solvents had to be dried by distillation under nitrogen and used as freshly prepared solvents. All other solvents were used as supplied without further purification.

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively (Bruker Biospin, USA). ^{1}H NMR chemical shifts are referenced to TMS or $CDCl_3$ (0; 7.26 ppm). ^{13}C NMR was referenced to $CDCl_3$ (77.0 ppm). Mass spectra and high-resolution mass spectra (HRMS) were measured using the electrospray ionization (ESI) technique (Bruker MicrOTOF, USA). Infrared spectra were performed by Fourier Transformed infrared spectrometer (Simadzu, Japan). Purification was carried out on column chromatography using silica gel 60 (Merck, 230–400 mesh). Chemical shifts are reported in part per million (ppm) and spectral data are represented in the following order: chemical shift; multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, m = multiplet); coupling constant (J, Hz); number of protons. Column chromatography was performed on silica gel Merck Kieselgel 70–230 mesh ASTM. Thin layer chromatography (TLC) was carried out on 5 × 20 cm plates with a layer thickness of 0.25 mm (Merck Silica gel 60 F_{254}). Elemental Analysis (C, H, N) of the target compounds are within ±0.4% of the calculated values unless otherwise noted.

4-(3-methoxyphenyl)isoquinoline (6)

To a solution of commercially available 4-bromoisoquinoline (0.080 g, 0.38 mmol) and 3-methoxyphenylboronic acid (0.087 g, 0.57 mmol) in 4 ml of DMF, the tetrakis(triphenylphosphine) palladium (0.022 g, 0.019 mmol) and an aqueous solution of sodium carbonate (0.12 g 1.14 mmol) were added and stirred. The reaction was then warmed at 65 °C for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (8:2) as eluent, to give 0.065 g of a colorless liquid (73%); Rf = 0.59 (Hexane/EtOAc 6:4); yield 73%; IR (KBr) 3053, 2937, 1602, 1484, 1226, 1160, 886 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.23 (s, 1H, H-1), 8.48 (s, 1H, H-3), 8.02 (d, J = 5.7 Hz, 1H, H-8), 7.93 (d, J = 6.0 Hz, 1H, H-5), 7.63 (m, 2H, H-6, H-7), 7.42 (t, J = 5.7 Hz, 1H, H-5′), 7.09–6.99 (m, 3H, H-2′, H-4′, H-6′), 3.85 (s, 3H, O-CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 159.7 (C-3′), 152.0 (C-1), 142.7 (C-1′), 138.3 (C-3), 134.2 (C-6), 130.6 (C-4), 129.6 (C-5′), 128.4 (C-4a), 127.9 (C-8), 127.2 (C-7), 124.8 (C-8a), 122.6 (C-5), 115.8 (C-6′), 113.4 (C-4′), 108.4 (C-2′), 55.3 (O-$\underline{C}H_3$); HRMS (ESI) *m/z* calculated: $C_{16}H_{13}NO$: 235.0997 found: 236.1076 $(M+H)^+$.

4-(4-(azepan-1-ylmethyl)phenyl)isoquinoline (12)

To a solution of commercially available 4-bromoisoquinoline (0.070 g, 0.34 mmol) and 4-(homopiperidine)methyl)phenylboronic acid (0.127 g, 0.40 mmol) in 4 ml of DMF, the tetrakis(triphenylphosphine) palladium (0.039 g, 0.017 mmol) and an aqueous solution of sodium carbonate (0.11 g 1.01 mmol) were added and stirred. The reaction was then warmed at 65 °C for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (7:3) as eluent, to give 0.083 g of a yellow liquid (78%); Rf = 0.29 (Hexane/EtOAc 8:2); IR (KBr) 3090, 2986, 2822, 1622, 1587, 1397, 1234 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.26 (s, 1H, H-1), 8.51 (s, 1H, H-3), 8.01 (d, J = 7.9, 1H, H-8), 7.66 (m, 3H, H-5, H-6, H-7), 7.50 (m, 4H, H-2′, H-3′), 3.77 (s, 2H, -Ph-CH_2-N), 2.72 (m, 4H, H-2″), 1.68 (m, 8H, CH_2-CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 151.9 (C-1), 142.8 (C-4′), 134.2 (C-3), 132.2 (C-6), 132.0 (C-4), 130.9 (C-4a), 130.5 (C-3′, C-5′), 130.0 (C-7), 129.2 (C-2′, C-6′), 128.4 (C-8), 127.9 (C-8a), 127.2 (C-1′), 124.9 (C-5), 62.3 (CH_2-N), 55.6 (C-1″), 27.8 (C-2″), 27.1 (C-3″); HRMS (ESI) *m/z* calculated: 316.1939 found: 317.2042 $(M+H)^+$.

4-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)isoquinoline (15)

To a solution of 4-ethynylisoquinoline (0.05 g, 0.33 mmol) and 1-azido-4-chlorobenzene (0.05 g, 0.33 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (6.5 mg, 0.033 mmol) and copper sulfate pentahydrate (0.8 mg, 0.0033 mmol) were added. The solution was stirred at room temperature for 48 h. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (8:2) as eluent, to give 0.042 g of a slightly yellow solid (42%); Rf = 0.15 (Hexane/EtOAc 7:3); m.p. 240–243 °C; IR (KBr) 3082, 3044, 1503, 1383, 1092, 990, 777 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.32 (s, 1H, H-1), 8.80 (s, 1H, H-3), 8.62 (d, J = 8.5, 1H,

H-8), 8.33 (S, 1H, H-5'), 8.09 (d, $J = 8.1$, 1H, H-5), 7.86–7.83 (m, 3H, H-6, H-2″, H-6″), 7.72 (m, 1H, H-7), 7.60 (m, 2H, H-3″, H-5″); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 153.4 (C-1), 143.0 (C-1′), 135.4 (C-3), 134.9 (C-1″), 133.7 (C- 4″), 133.6 (C- 5′), 131.4 (C-4), 130.1 (C-6), 128.5 (C-4a), 128.1 (C-3″, C-5″), 127.7 (C-8), 124.4 (C-7), 121.8 (C-8a), 121.4 (C-2″, C-6″), 120.5 (C-5); HRMS (ESI) *m/z* calculated: 306.0672 found: 307.0750 $(M+H)^+$.

4-(1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)isoquinoline (16)

To a solution of 4-ethynylisoquinoline (0.05 g, 0.33 mmol) and 1-azido-3-chlorobenzene (0.05 g, 0.33 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (6.5 mg, 0.033 mmol) and copper sulfate pentahydrate (0.8 mg, 0.0033 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (8:2) as eluent, to give 0.073 g of a yellow solid (73%); Rf = 0.09 (Hexane/EtOAc 8:2); m.p. 236–240 °C; IR (KBr) 3119, 1624, 1599, 1499, 1057, 798 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.32 (s, 1H, H, H-1), 8.81 (s, 1H, H-3), 8.61 (d, $J = 8.6$ Hz, 1H, H-8), 8.35 (s, 1H, H-5′), 8.10 (d, $J = 8.1$, 1H, H-5), 7.94 (m, 1H, H-2″), 7.86–7.79 (m, 2H, H-6, H-6″), 7.72 (m, 1H,H-7), 7.58 (t, $J = 7.6$, 1H, H-5″), 7.52 (m, 1H, H-4″); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 153.4 (C-1), 145.4 (C-1′), 143.0 (C-3), 137.7 (C-1″), 135.8 (C-3″), 133.6 (C-5′), 131.4 (C-4), 131.0 (C-5″), 129.1 (C-6), 128.6 (C-4a), 128.1 (C-4″), 127.7 (C-8), 124.8 (C-7), 121.3 (C-6″), 120.9 (C-2″), 120.5 (C-8a), 118.6 (C-5); HRMS (ESI) *m/z* calculated: 306.0672 found:307.0749 $(M+H)^+$.

3-(4-(isoquinolin-4-yl)-1H-1,2,3-triazol-1-yl)benzonitrile (19)

To a solution of 4-ethynylisoquinoline (0.05 g, 0.33 mmol) and 3-azidobenzonitrile (0.047 g, 0.33 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (6.5 mg, 0.033 mmol) and copper sulfate pentahydrate (0.8 mg, 0.0033 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (8:2) as eluent, to give 0.085 g of a white solid (88%); Rf = 0.17 (Hexane/EtOAc 7:3); m.p. 247–249 °C; IR (KBr) 3057, 2228, 1595, 1420, 1045, 798 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.34 (s, 1H, H-1), 8.82 (s, 1H, H-3), 8.59 (d, $J = 8.5$ Hz, 1H, H-8) 8.40 (s, 1H, H-5′), 8.24 (m, 1H, H-6″), 8.20 (m, 1H, H-4″), 8.11 (d, $J = 8.1$ Hz, 1H, H-5), 7.81–7.88 (m, 2H, H-6, H-2″), 7.79 (d, $J = 7.8$ Hz, 1H, H-5″) 7.74 (m, 1H, H-7); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 153.5 (C-1), 145.8 (C-1′), 142.9 (C-3), 137.4 (C-6″), 133.2 (C-2″), 132.4 (C-4″), 131.6 (C-5′), 131.1 (C-6), 128.6 (C-4), 128.2 (C-5″), 127.8 (C-4a), 124.8 (C-1″), 124.6 (C-8), 123.7 (C-7), 121.3 (C-8a), 120.3 (C-5), 117.3 (C≡N), 114.3 (C-3″); MS (ESI) *m/z* calculated: 297.1014 found: 298.1089 $(M+H)^+$.

4-(1-benzhydryl-1H-1,2,3-triazol-4-yl)isoquinoline (25)

To a solution of 4-ethynylisoquinoline (0.05 g, 0.33 mmol) and (azidomethylene)dibenzene (0.068 g, 0.33 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (6.5 mg, 0.033 mmol) and copper sulfate pentahydrate (0.8 mg, 0.0033 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (7:3) as eluent, to give 0.046 g of a white solid (39%); Rf = 0.20 (Hexane/EtOAc 7:3); m.p. 139–143 °C; IR (KBr) 3088, 3030, 1495, 1227, 1057, 901, 750 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 9.25 (S, 1H, H-1), 8.68 (s, 1H, H-3), 8.62 (d, $J = 8.5$ Hz, 1H, H8), 8.04 (d, $J = 8.0$ Hz, 1H, H-5), 7.84–7.74 (m, 2H, H-6, H-5′), 7.68 (dd, $J = 8.0$, 1.1 Hz, 1H, H-7), 7.57–7.32 (m, 6H, H-3″, H-4″, H-5″), 7.57–7.32 (m, 5H, H-2″, H-6″, CH); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 152.4 (C-1), 144.1 (C-1′), 141.9 (C-3), 138.0 (C-1″), 133.7 (C-6), 131.6 (C-5′), 129.1 (2, C-3″, C4a), 128.8 (C-4a), 128.1 (2, C-8, C-2″), 127.8 (C-7), 125.2 (C-4″), 122.7 (C-8a), 122.2 (C-5), 68.4 (CH); MS (ESI) *m/z* calculated: 362.1531 found: 363.1609 $(M+H)^+$.

1-(4-(tert-butyl)benzyl)-4-(naphthalen-1-yl)-1H-1,2,3-triazole (27)

To a solution of 4-ethynylisoquinoline (0.02 g, 0.131 mmol) and 1-(azidomethyl)-4-(tert-butyl)benzene (0.025 g, 0.131 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (2.6 mg, 0.013 mmol) and copper sulfate pentahydrate (0.3 mg, 0.0013 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (8:2) as eluent, to give 0.015 g of an amorphous white solid (33%); Rf = 0.33 (Hexane/EtOAc 8:2); m.p. 158–160 °C; IR (KBr)

3119, 2963, 1458, 1360, 1221, 1053, 980, cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.36 (m, 1H, H-5), 7.85 (m, 2H, H-1, 8), 7.71–7.66 (m, 2H, H-5', H-3), 7.54–7.47 (m, 3H, H-2, 6, 7), 7.41 (d, $J = 8.0$ Hz, 2H, H-3″, H-5″), 7.29 (d, $J = 8.0$ Hz, 2H, H-2″, H-6″), 5.58 (s, 2H, CH_2), 1.30 (s, 9H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 152.0 (C-4″), 147.3 (C-1′), 133.9 (C-4), 131.6 (C-8a), 131.1 (C-1″), 128.9 (C-5a), 128.4 (C-5′), 128.0 (C-8), 127.2 (C-5), 126.6 (C-1), 126.1 (C-7), 126.0 (C-2″), 125.5 (C-6), 125.3 (C-2), 124.4 (C-3″), 122.4 (C-3), 54.0 (CH_2), 34.7 (CH), 31.3 (CH_3); MS (ESI) m/z calculated: 341.1892 found:364.1785 $(M+Na)^+$.

1-(4-chlorophenyl)-4-(naphthalen-1-yl)-1H-1,2,3-triazole (28)

To a solution of 1-ethynylnaphthalene (0.02 g, 0.128 mmol) and 1-azido-4-chlorobenzene (0.02 g, 0.128 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (2.5 mg, 0.013 mmol) and copper sulfate pentahydrate (0.3 mg, 0.0013 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (8:2) as eluent, to give 0.032 g of a yellow solid (83%); Rf = 0.40 (Hexane/EtOAc 8:2); m.p. 149–151 °C; IR (KBr) 3125, 3063, 1593, 1500, 1233, 1049, 829 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.40 (m, 1H, H-5), 8.21 (s, 1H, H-5′), 7.90 (m, 2H, H-1, H-8), 7.79 (m, 3H, H-3, H-2"), 7.55 (m, 4H, H-6, H-7, H-3″, H-5″); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 147.8 (C-1′), 135.6 (C-4), 134.6 (C-1″), 133.9 (C-4″), 131.1 (C-8a), 130.0 (C-4a), 129.3 (C-5'), 128.6 (C-3″), 127.4 (C-5), 126.9 (C-1), 126.2 (C-8), 125.4 (C-7), 125.3 (C-6), 123.8 (C-2), 121.7 (C-3), 120.4 (C-2″); MS (ESI) m/z calculated: 305.0720 found: 328.0626 $(M+Na)^+$.

1-benzhydryl-4-(naphthalen-1-yl)-1H-1,2,3-triazole (29)

To a solution of 1-ethynylnaphthalene (0.024 g, 0.154 mmol) and (azidomethylene)dibenzene (0.032 g, 0.154 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (3.1 mg, 0.015 mmol) and copper sulfate pentahydrate (0.4 mg, 0.0015 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ethyl acetate (9:1) as eluent, to give 0.017 g of a white solid (30%); Rf = 0.39 (Hexane/EtOAc 8:2); m.p. 161–163 °C; IR (KBr) 3088, 3057, 1599, 1498, 1450, 1051 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.37 (m, 1H, H-5), 7.86 (m, 2H, H-1, H-8), 7.68 (m, 2H, H-2, H-3), 7.48 (m, 3H, H-1′, H-6, H-7), 7.39 (m, 6H, H-3″, H-4″, H-5″), 7.21 (m, 5H, H-2″, CH); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 146.7 (C-1′), 138.2 (C-4), 133.9 (C-1′), 130.1 (C-8a), 129.0 (C-3″), 128.9 (C-5′), 128.7 (C-4a), 128.5 (C-5), 128.2 (C-2′), 127.8 (C-8), 127.3 (C-1), 126.7 (C-6), 126.0 (C-7), 125.5 (C-4′), 125.3 (C2), 122.3 (C-3), 68.3 (CH); MS (ESI) *m/z* calculated: 361.1579 found: 384.1488 $(M+Na)^+$.

4-(1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazol-4-yl)-7-chloroquinoline (30)

To a solution of 7-chloro-4-ethynylquinoline (0.08 g, 0.49 mmol) and 1-(azidomethyl)-4-(tert-butyl)benzene (0.092 g, 0.49 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (9.7 mg, 0.049 mmol) and copper sulfate pentahydrate (1.2 mg, 0.0049 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (9:1) as eluent, to give 0.068 g of a colorless liquid; (39%); Rf = 0.17 (Hexane/EtOAc 9:1); IR (KBr) 3121, 2965, 1605, 1458, 1219, 1047, 991, 723 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.79 (d, $J = 4.4$ Hz, 1H, H-2), 8.55 (d, $J = 9.1$ Hz, 1H, H-5), 8.05 (s, 1H, H-8), 7.90 (s, 1H, H-5′), 7.46 (d, J = 4.4 Hz, 1H, H-6), 7.44–7.33 (m, 3H, H-3, H-3″, H-5″), 7.25 (d, $J = 8.1$ Hz, 2H, H-2″, H-6″), 5.57 (s, 2H, CH_2), 1.26 (s, 9H, 3xCH_3); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 152.3 (C-2), 150.8 (C-4″), 149.1 (C-8a), 144.9 (C-1′), 136.3 (C-4), 135.6 (C-7), 131.1 (C-1″), 128.5 (C-5'), 128.1 (2) (C-5, C-8), 127.5 (C-6), 126.3 (C-2″), 123.9 (C-3″), 123.5 (C-4a), 120.3 (C-3), 54.2 (CH_2), 34.7 (CH), 31.2 (CH_3); MS (ESI) m/z calculated: 376.1455 found: 377.1537 $(M+H)^+$.

7-chloro-4-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl) quinoline (31)

To a solution of 7-chloro-4-ethynylquinoline (0.08 g, 0.49 mmol) and 1-azido-4-chlorobenzene (0.075 g, 0.49 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (9.7 mg, 0.049 mmol) and copper sulfate pentahydrate (1.2 mg, 0.0049 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (9:1) as

eluent, to give 0.066 g of an orange solid (39%); Rf = 0.20 (Hexane/EtOAc 7:3); m.p. 161–164 °C;IR (KBr) 3125, 2957, 1593, 1499, 1391, 1230, 1047, 991, 827 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, 25 °C) δ 9.61 (s, 1H, H-5″), 9.06 (d, J = 4.4 Hz, 1H, H-2), 8.90 (d, J = 9.1 Hz, 1H, H-5), 8.18 (s, 1H, H-8), 8.09 (d, J = 8.7 Hz, 2H, H-2″, H-6″), 7.94 (d, J = 4.4 Hz, 1H, H-6), 7.74 (m, 3H, H-3, H-3″, H-5″); ^{13}C-NMR (75 MHz, $CDCl_3$, DMSO-d_6, 25 °C) δ 150.7 (C-2), 150.0 (C-1′), 149.9 (C-8a), 139.4 (C-4), 136.0 (C-7), 135.5 (C-1″), 134.7 (C-4″), 129.9 (C-5′), 128.9 (C-5), 128.3 (C-8), 127.8 (C-3″), 125.3 (C-6), 122.6 (C-2″), 121.7 (C-4a), 120.3 (C-3); MS (ESI) m/z calculated: 340.0283 found: 341.0363 $(M+H)^+$.

4-(1-benzhydryl-1H-1,2,3-triazol-4-yl)-7-chloroquinoline (32)

To a solution of 7-chloro-4-ethynylquinoline (0.08 g, 0.49 mmol) and (azidomethylene)dibenzene (0.102 g, 0.49 mmol) in a mixture of *tert*-butanol (1:1, 0.5 mL). Sodium ascorbate (9.7 mg, 0.049 mmol) and copper sulfate pentahydrate (1.2 mg, 0.0049 mmol) were added. The solution was stirred at room temperature for overnight. The mixture was diluted with water and extracted with ethyl acetate (×3). The combined organic fractions were washed with brine (×1), dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography by using hexane/ ethyl acetate (9:1) as eluent, to give 0.062 g of an yellow solid (32%); Rf = 0.20 (Hexane/EtOAc 7:3); m.p. 168–170 °C; IR (KBr) 3092, 3045, 2955, 1587, 1493, 1049, 878 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.88 (d, *J* = 4.3 Hz, 1H, H-2), 8.65 (d, J = 9.1 Hz, 1H, H-5), 8.13 (s, 1H, H-8), 7.83 (s, 1H, H-1′), 7.53 (m, 2H, H-3, H-6), 7.39 (m, 6H, 2 H-3″, 2 H-4″, 2 H-5″), 7.20 (m, 5H, CH, 2 H-2″, 2 H-6″); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C) δ 149.9 (C-2), 148.2 (C-8a), 144.4 (C-1′), 137.6 (C-4), 137.2 (C-1″), 136.4 (C-7), 129.2 (C5′), 129.0 (C-3″), 128.7 (C-5), 128.1 (C-8), 127.9 (C-6), 127.8 (C-2″), 124.0 (C-4″), 123.8 (C-4a), 120.2 (C-3), 68.6 (CH); MS (ESI) m/z calculated: 396.1142 found: 397.1230 $(M+H)^+$.

Biological assay

Assessment of in vitro antimalarial activity of synthetic compounds

All synthesized compounds were evaluated for their in vitro antiplasmodial activity against the *P. falciparum* chloroquine-sensitive (3D7) and chloroquine-resistant (K1) laboratory strains. The parasite was briefly cultured according to the method laid out in [27], in O^+ human erythrocytes suspended in an RPMI culture medium supplemented with 10% human B serum, 25 mM HEPES and 15 mg/ml gentamicin at 37 °C in a 5% CO_2, 5% O_2, and 90% N_2 atmosphere. The parasite was maintained at 5% parasitemia and subcultured every 48 h. Antimalarial activity of the synthetic compounds was assessed using SYBR Green I assay [28, 29]. The parasites were synchonized by 5% sorbitol to obtain the ring stage of *P. falciparum*. The highly synchronous ring stage parasite culture was prepared to a parasitemia of 2% and hematocrit of 1%. The tested compound was dissolved in DMSO (vehicle) and diluted for 50 times with the complete media to obtain the first working concentration containing 2% DMSO. The 50 µL of parasite mixture was inoculated into the 50 µL predosed compounds in 96-well plates. The 100 µL final volume contained a parasite with a parasitemia of 1% and hematocrit of 0.5%, and maximum DMSO (vehicle) at 1% for the highest compound concentration. The parasite mixture was exposed to the following eight levels of concentration as testing compounds: 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 µM (at 37 °C in a 5% CO_2, 5% O_2, and 90% N_2 atmosphere) for 48 h. The parasite DNA content was stained by SYBR green I diluted in lysis buffer, and fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 485 nm and 530 nm, respectively. The experiment was repeated three times and triplicated for each experiment. The IC_{50} values (concentrations that inhibit the parasite growth by 50%) were calculated from a log-dose–response curve plotted using the Calcusyn™ version 1.1 (BioSoft, Cambridge, UK) and used as indicators of antimalarial activity. Four standard antimalarial drugs (chloroquine, CQ; quinine, Q; mefloquine, MQ and dihydroartemisinin, DHA) were used as a positive control.

Observation of morphological change in cells following exposure to the selected synthetic compounds

Synchronized *P. falciparum* 3D7 and K1 strains were used in the experiment. The parasites were exposed to the separated selected synthetic compounds and two standard antimalarial drugs, chloroquine and DHA, which were used as a positive control at IC_{100}. The treated parasites were incubated in a 5% CO_2, 5% O_2, and 90% N_2 atmosphere at 37 °C for 72 h. Blood films were prepared at the following time points: 6, 12, 24, 36, 48, 60, 72 h and stained with Giemsa (Biotechnical Thai, Bangkok, Thailand). The morphology of the resulting parasites was observed and captured under a light microscope (×100, Leica Microsystems, Hesse, Germany) [30].

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Author contributions ST participated in design of study, provided all synthesized compounds and elucidated structure of all compounds and interpret data, PM carried out the main biological experiment, participated in image and performed the statistical analysis, AT assisted in some biological study, OV helped to interpret the data and gave comment in experiment and paper. ST and PM helped to draft the paper. ST read and approved the final paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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