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New compounds hybrids 1*H*-1,2,3-triazole-quinoline against *Plasmodium falciparum*

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Malaria is one of the most prevalent parasitic diseases in the world. The global importance of this disease, current vector control limitations and the absence of an effective vaccine make the use of therapeutic antimalarial drugs the main strategy to control malaria. Chloroquine (CQ) is a cost effective antimalarial drug with a relatively robust safety profile, or therapeutic index. However, CQ is no longer used alone to treat patients with *Plasmodium falciparum* due to the emergence and spread of CQ-resistant strains, which have also been reported for *Plasmodium vivax*. However, the activity of 1,2,3-triazole derivatives against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* has been reported in the literature. To enhance the anti-*P. falciparum* activity of quinoline derivatives, we synthesized 11 new quinoline-1*H*-1,2,3-triazole hybrids with different substituents in the 4-positions of the 1*H*-1,2,3-triazole ring, which were assayed against the W2-chloroquine-resistant *P. falciparum* clone. Six compounds exhibited activity against the *P. falciparum* W2 clone, chloroquine-resistant, with IC_{50}

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values ranging from 1.4 to 46 μM. None of these compounds was toxic to a normal monkey kidney cell line (BGM), thus exhibiting good selectivity indexes, as high 351 for one compound (**11**).

Introduction

Malaria is one of the most prevalent parasitic diseases in the world and affects approximately 500 million individuals throughout the tropical and subtropical areas of developing countries. Malaria causes considerable morbidity and mortality and is associated with approximately 800,000 deaths worldwide per year[1].

The global importance of this disease, current vector control limitations and the absence of an effective vaccine make the development of new therapeutic drugs, since the main strategy used to control malaria is patients treatment with antimalarials[2]. Chloroquine (CQ) is a cost effective antimalarial drug with a relatively robust safety profile, or therapeutic index[3]. However, CQ is no longer used alone to treat patients with *Plasmodium falciparum* due to the emergence and spread of CQ-resistant strains, which have also been reported for *P. vivax*[4,5].

Although many new compounds have been described in the recent literature, the quinoline derivatives continue to dominate the antimalarial drug category[6-8]. Many of the studies have been conducted using synthetic hybrid compounds that were designed to increase the efficacy of quinoline derivatives, such as AZT-chloroquinoline (**I**)[9], quinine-dihydroartemisinin (**II**)[10] and MEFAS (**III**)[11], a salt derived from mefloquine and artesunate.

The activity of 1,2,3-triazole derivatives I**V-VI** against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* has also been described[12-14] (**Figure 1**).

During our search for new prototypes that can be used to treat neglected diseases, we have demonstrated the importance of the 1,2,3-triazoles against *Mycobacterium tuberculosis*[15] and *Leishmania amazonensis*[16]. In an effort to find new prototypes active against *P. falciparum* we have synthesized new quinoline-1*H*-1,2,3-triazole hybrids (**3**-**13**). The 7-chloroquinoline moiety was included in these hybrids because it is present in CQ and amodiaquine, two drugs that are commonly used to treat malaria. While the 1*H*-1,2,3-triazoles contain a variety of substituents at the 4-position, were chosen based on its activities against *P. falciparum*[12-14] (**Figure 2**).

Materials and methods

Chemistry

The ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were generated at 400.00, 100.00 and 376.00 MHz, respectively, at 25 ^{o}C, using a BRUKER Avance instrument equipped with a 5 mm probe. Tetramethylsilane was used as an internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants (*J*) are reported in Hertz. The Fourier transform infrared (FTIR) absorption spectra were recorded on a Shimadzu mode IR Prestige-21 spectrophotometer through KBr

reflectance. The melting points (m.p.) were determined using a Büchi model B-545 apparatus. TLC (thin layer chromatography) was performed using a Merck TLC Silica gel 60 F_{254} aluminium sheets 20 x 20 cm (eluent hexane/ethylacetate 2:8). Gravitational column chromatography was performed using Silica gel 60 (0.2-0.5 mm) the gradient mixture initial hexane/ethyl acetate 100-0% until 0-100%, volume of gradient 100mL, column dimensions 25 cm x 3.5 cm. All other reagents and solvents used were analytical grade.

General procedure for the preparation of 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines (3-10).

A mixture of H_2O/t-BuOH (1:1) (40 mL) was added to 4-azido-7-chloroquinoline **(2)** (22.5 mmol), the appropriate acetylene (33.7 mmol), L-ascorbic acid sodium salt (2.25 mmol) and $CuSO_4.5H_2O$ (0.225) mmol. The reaction mixture was maintained under vigorous stirring at 25 °C for 24 h. The progress of the reaction was monitored using TLC (hexane/ethylacetate 2:8). On completion, the reaction mixture was poured in to ice cold water (50 mL). The precipitate was filtered and dried. The residual crude product was purified via silica gel column chromatography using the gradient mixture initial hexane/ethyl acetate 100-0% until 0-100% to produce 86-31% yields of the pure derivatives **3**-**10**.

7-chloro-4-(4-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)quinoline **(3)**

Yield: 77 %. m.p. 93.2 – 94.0 °C. IR (KBr, cm^{-1}): 3446; 2914; 1637; 1612; 1560; 1506; 1118; 1026; 817; 624. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.48-3.85 (m, 8H,); 4.69 (d, *J*= 12.3 Hz, 1H, H2'); 4.81 (s, 1H, CH_2O); 4.86 (d, *J*= 12.3 Hz, 1H, H2'); 7.80 (dd, *J*= 2.0, 9.0 Hz, 1H, H-6); 7.87 (d, *J*= 4.6 Hz, 1H, H-3); 8.03 (d, *J*= 9.0 Hz, 1H, H-5); 8.29 (d, *J*= 2.0 Hz, 1H, H-8); 8.84 (s, 1H, H-triazole); 9.16 (d, 1H, *J*= 4.6 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.9; 24.9; 30.0; 59.2; 61.3; 97.2; 117.0; 120.3; 125.5; 126.2; 128.0; 128.9; 135.3; 140.4; 144.8; 149.3; 152.3. Anal. Calcd for $C_{17}H_{17}ClN_4O_2$: C, 59.22; H, 4.97; N, 16.25. Found: C, 58.86; H, 5.06; N, 15.92.

1-(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)cyclohexanol **(4)**

Yield: 65 %. m.p. 158.1 – 160.0 °C. IR (KBr, cm^{-1}): 3446; 3149; 1637; 1614; 1564; 1508; 1319; 1031; 808. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.33-2.04 (m, 10H, cyclohex); 5.11 (s, 1H, OH); 7.80 (dd, *J*= 2.1, 9.1 Hz, 1H, H-6); 7.82 (d, 1H, *J*= 4.6 Hz, H-3); 8.06 (d, *J*= 9.1 Hz, 1H, H-5); 8.27 (d, *J*= 2.1 Hz, 1H, H-8); 8.62 (1H, s, H-triazole); 9.13 (1H, d, *J*= 4.6 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.6; 25.1; 37.6; 67.9; 116.7; 120.2; 123.3; 125.5; 128.1; 128.8; 135.2; 140.5; 149.4; 152.3; 156.5. Anal. Calcd for $C_{17}H_{17}ClN_4O$: C, 62.10; H, 5.21; N, 17.04. Found: C, 62.27; H, 5.31; N, 16.63.

7-chloro-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)quinoline **(5)**

Yield: 55 %. m.p. 148.5 – 149.2 °C. IR (KBr, cm^{-1}): 3446; 3124; 3051; 2357; 1681; 1608; 1558; 1506; 1456; 1435; 1234; 1022; 877; 767. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.43 (t, *J*= 7.4 Hz, 1H, H-Ph); 7.54 (t, *J*= 7.4 Hz, 2H, H-Ph); 7.81 (dd, 1H, *J*= 2.0, 9.1

Hz, H-6); 7.95 (d, 1H, *J*= 4.6 Hz, H-3); 8.01 (d, *J*= 7.2 Hz, 2H, H-Ph); 8.16 (d, *J*= 9.1 Hz, 1H, H-5); 8.31 (d, *J*= 2.0 Hz, 1H, H-8); 9.21 (d, *J*= 4.6 Hz, 1H, H-2) 9.33 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.8; 120.1; 123.6; 125.5; 125.6; 128.0; 128.4; 128.9; 129.0; 129.7; 135.3; 140.3; 147.0; 149.4; 152.3. Anal. Calcd for $C_{17}H_{11}ClN_4$: C, 66.56; H, 3.61; N, 18.26. Found: C, 66.02; H, 3.44; N, 18.02.

7-chloro-4-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)quinoline **(6)**

Yield: 40 %. m.p. 147.6 – 149.1 °C. IR (KBr, cm^{-1}): 3446; 3124; 3051; 2357; 1681; 1608; 1558; 1506; 1456; 1435; 1234; 1022; 877; 767. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87-0.91 (m, 2H, *c*-prop); 1.00-1.05 (m, 2H, *c*-prop); 2.09-2.14 (m, 1H, *c*-prop); 7.78 (dd, 1H, *J*= 2.0, 9.0 Hz, H-6); 7.80 (d, 1H, *J*= 4.6 Hz, H-3); 8.08 (d, 1H, *J*= 9.0 Hz, H-5); 8.27 (d, 1H, *J*= 2.0 Hz, H-8); 8.57 (s, 1H, H-triazole); 9.13 (d, 1H, *J*= 4.6 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 6.41; 7.84; 116.5; 120.1; 122.9; 125.6; 128.0; 128.8; 135.2; 140.9; 149.4; 149.9; 152.2. Anal. Calcd for $C_{17}H_{11}ClN_4$: C, 62.11; H, 4.10; N, 20.70. Found: C, 61.93; H, 3.92; N, 20.62.

ethyl 1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazole-4-carboxylate **(7)**

Yield: 55 %. m.p. 137.4 – 139.0 °C. IR (KBr, cm^{-1}): 3448; 3232; 1942; 1722; 1541; 1506; 1265; 1220; 1155; 1033; 825; 771. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.36 (t, *J*= 7.0 Hz, 3H, CH_3); 4.40 (q, *J*= 7.0 Hz, 2H, CH_2); 7.79 (dd, *J*= 1.2, 9.0 Hz, 1H, H-6); 7.94 (d, *J*= 3.4 Hz, 1H, H-3); 7.95 (d, *J*= 9.0 Hz, 1H, H-5); 8.31 (d, *J*= 1.2 Hz, 1H, H-8); 9.20 (d, *J*= 3.4 Hz, 1H, H-2); 9.48 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.1; 60.8; 117.0; 120.3; 125.2; 128.0; 129.1; 131.3; 135.4; 139.4; 139.8; 149.2; 152.2; 159.8. Anal. Calcd for $C_{14}H_{11}ClN_4O_2$: C, 55.55; H, 3.66; N, 18.51. Found: C, 55.64; H, 3.99; N, 17.98.

methyl 1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazole-4-carboxylate **(8)**

Yield: 40 %. m.p. 183.7 – 185.0 °C. IR (KBr, cm^{-1}): 3444; 3120; 3064; 1919; 1707; 1614; 1550; 1508; 1436; 1344; 1280; 1220; 1159; 1033; 815; 779. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.93 (s, 3H, CH_3); 7.80 (dd, *J*= 2.1, 9.0 Hz, 1H, H-6); 7.94 (d, *J*= 4.6 Hz, 1H, H-3); 7.95 (d, *J*= 9.0 Hz, 1H, H-5); 8.31 (d, *J*= 2.1 Hz, 1H, H-8); 9.19 (d, *J*= 4.6 Hz, 1H, H-2); 9.51 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 52.1; 117.0; 120.3; 125.2; 128.0; 129.1; 131.3; 135.4; 139.4; 139.8; 149.2; 152.3; 160.3. Anal. Calcd for $C_{13}H_9ClN_4O_2$: C, 54.09; H, 3.14; N, 19.41. Found: C, 54.23; H, 3.36; N, 18.17.

2-(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)propan-2-ol **(9)**

Yield: 71 %. m.p. 143.3 – 144.8 °C. IR (KBr, cm^{-1}): 3441; 3159; 3035; 1961; 1633; 1560; 1504; 1435; 1361; 1176; 1033; 808; 773. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.60 (s, 6H, CH_3); 5.35 (s, 1H, OH); 7.80 (dd, *J*= 2.0, 9.0 Hz, 1H, H-6); 7.83 (d, *J*= 4.6 Hz, 1H, H-3); 8.06 (d, *J*= 9.0 Hz, 1H, H-5); 8.27 (d, *J*= 2.0 Hz, 1H, H-8); 8.61 (s, 1H, H-triazole); 9.14 (d, *J*= 4.6 Hz, 1H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.5; 67.0; 116.7; 120.3; 122.5; 125.5; 128.1; 128.8; 135.2; 140.5; 149.4; 152.3; 156.6. Anal. Calcd for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40. Found: C, 58.26; H, 4.54; N, 19.42.

(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methanol **(10)**

Yield: 45 %. m.p. 161.1 – 163.0 °C. IR (KBr, cm^{-1}): 3097; 2883; 2821; 1915; 1608; 1589; 1562; 1504; 1436; 1350; 1307; 1273; 1240; 1120; 1078; 1043; 821; 771. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.71 (d, *J*= 5.6 Hz, 2H, CH_2); 5.42 (t, *J*= 5.6 Hz, 1H, OH); 7.80 (dd, *J*= 2.0, 9.0 Hz, 1H, H-6); 7.83 (d, *J*= 4.6 Hz, 1H, H-3); 8.03 (d, *J*= 9.0 Hz, 1H, H-5); 8.28 (d, *J*= 2.0 Hz, 1H, H-8); 8.70 (s, 1H, H-triazole); 9.15 (d, *J*= 4.6 Hz, 1H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 54.8; 116.9; 120.3; 125.0; 125.4; 128.1; 128.9; 135.3; 140.5; 148.8; 149.3; 152.3. Anal. Calcd for $C_{12}H_9ClN_4O$: C, 55.29; H, 3.48; N, 21.49. Found: C, 55.29; H, 3.35; N, 21.21.

Procedure for the preparation of 1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazole-4-carbaldehyde (11)

A solution of 0,4 mL (4.08 mmol) of freshly distilled oxalyl chloride and 4 mL of dry CH_2Cl_2 was added in a dropwise fashion to a solution of 0,6 mL (8.17 mmol) of dry DMSO in CH_2Cl_2 (2 mL) at -78 °C under Ar. After 15 min, a solution of 0.36 g (1.36 mmol) of compound 10 in 15 mL of CH_2Cl_2 was added. After 15 min, 1.5 mL (10.2 mmol) of triethylamine was added in a dropwise fashion at such a rate that the temperature remained at -78 °C. The mixture was warmed to 25 °C for 2 hours. Then, 15 mL of cold water was added and neutralized with 5% NaOH. The mixture was separated, and the aqueous layer was extracted using chloroform (3 x 30 mL). The combined organic layers was washed with water, dried over $MgSO_4$, filtered, and concentrated. The residual crude product was purified via silica gel column chromatography using the gradient mixture initial hexane/ethyl acetate 100-0% until 0-100% to produce the pure derivative **11**.

Yield: 55 %. m.p. 187.9 – 189.0 °C. IR (KBr, cm^{-1}): 3448; 3118; 3032; 1924; 1697; 1612; 1533; 1506; 1440; 1344; 1263; 1205; 1112; 1033; 813; 771. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.81 (dd, *J*= 1.8, 9.0 Hz, 1H, H-6); 7.95 (d, *J*= 4.6 Hz, 1H, H-3); 7.96 (d, *J*= 9.0 Hz, 1H, H-5); 8.33 (d, *J*= 1.8 Hz, 1H, H-8); 9.21 (d, *J*= 4.6 Hz, 1H, H-2); 9.59 (s, 1H, H-triazole); 10.21 (s, 1H, CHO). ^{13}C RMN (DMSO-d_6, 100 MHz) δ: 117.8 120.3; 125.2; 128.1; 129.2; 130.6; 135.5; 139.8; 147.1; 149.2; 152.3; 185.0. Anal. Calcd for $C_{12}H_7ClN_4O$: C, 55.72; H, 2.73; N, 21.66. Found: C, 54.78; H, 2.51; N, 20.93.

Procedure for the preparation of 7-chloro-4-(4-(fluoromethyl)-1*H*-1,2,3-triazol-1-yl)quinoline (12)

The reaction mixture of alcohol derivative 10 (1 mmol), DAST (1 mmol), and CH_2Cl_2 (20 mL) was stirred at room temperature for 24 h and then poured into ice cold water. The mixture was extracted using chloroform (3 x 30 mL). The combined organic layers was washed with water, dried over $MgSO_4$, filtered, and concentrated. The residual crude product was purified via silica gel column chromatography using the gradient mixture initial hexane/ethyl acetate 100-0% until 0-100% to produce the pure derivative **12**.

Yield: 70 %. m.p. 159.4 – 160.0 °C. IR (KBr, cm^{-1}): 3442; 3140; 3066; 1942; 1749; 1610; 1560; 1502; 1454; 1379; 1340; 1305; 1255; 1234; 1205; 1118; 1051; 1016; 977; 815; 765. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.65 (d, *J*= 48.48 Hz, 2H, CH_2F); 7.80 (dd, J_1= 2.1, 9.1 Hz, 1H, H-6); 7.89 (d, *J*= 4.6 Hz, 1H, H-3); 7.98 (d, *J*= 9.1 Hz, 1H, H-5); 8.31 (d, *J*= 2.1 Hz, 1H, H-8); 9.05 (d, *J*= 3.1 Hz, 1H, H-triazole); 9.18 (d, *J*= 4.6 Hz,

1H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 75.0 (d, *J*= 159.8 Hz, CH_2F); 117.2; 120.2; 125.2; 127.7 (d, *J*= 4.19 Hz, CH-triazole); 128.0; 129.0; 135.3; 140.1; 142.6 (d, *J*= 20.0 Hz Cq-triazole); 149.2; 152.2. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -204.22 (s, 1F, CH_2F). Anal. Calcd for $C_{12}H_8ClFN_4$: C, 54.87; H, 3.07; N, 21.33. Found: C, 54.81; H, 3.11; N, 20.81.

Procedure for the preparation of 7-chloro-4-(4-(difluoromethyl)-1*H*-1,2,3-triazol-1-yl)quinoline (13)

The reaction mixture of aldehyde derivative 11 (1 mmol), DAST (15 mmol), and CH_2Cl_2 (20 mL) was stirred at room temperature for 24 h and then poured into ice cold water. The mixture was extracted using chloroform (3 x 30 mL). The combined organic layers was washed with water, dried over $MgSO_4$, filtered, and concentrated. The residual crude product was purified via silica gel column chromatography using the gradient mixture initial hexane/ethyl acetate 100-0% until 0-100% to produce the pure derivative **13**.

Yield: 60 %. m.p. 139.4 – 140.6 °C. IR (KBr, cm^{-1}): 3421; 1921; 1743; 1612; 1558; 1504; 1454; 1438; 1321; 1257; 1120; 1091; 1029; 823; 796. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.41 (t, *J*= 53.9 Hz, 1H, CHF_2); 7.80 (dd, *J*= 2.0, 9.0 Hz, 1H, H-6); 7.92 (d, *J*= 4.6 Hz, 1H, H-3); 7.93 (d, *J*= 9.0 Hz, 1H, H-5); 8.30 (d, *J*= 2.0 Hz, 1H, H-8); 9.18 (d, *J*= 4.6 Hz, 1H, H-2) 9.26 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 110.0 (t, *J*= 231.6 Hz CHF_2); 117.6; 120.3; 125.2; 127.0 (t, *J*= 3.14 Hz, CH-triazole); 128.1; 129.2; 135.5; 140.0; 142.0 (t, *J*= 27.4 Hz, Cq-triazole); 149.3; 152.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -112.56 (s, 2F, CHF_2). Anal. Calcd for $C_{12}H_7ClF_2N_4$: C, 51.35; H, 2.51; N, 19.96. Found: C, 51.01; H, 2.58; N, 19.27.

Biological evaluation

Continuous cultures and in vitro assays using *P. falciparum*-infected erythrocytes

The *P. falciparum* W2 clone, chloroquine-resistant and mefloquine-sensitive[17], was maintained in continuous culture as previously described[18], at 37 °C in human erythrocytes (A^+) in complete medium (RPMI 1640 supplemented with 10% human sera blood group A^+, 2% glutamine and 7.5% $NaHCO_3$). Cultures in Petri dishes placed either in a candle jar, or 25 cm culture flasks in an environment containing a gas mixture atmosphere (3% O_2, 5% CO_2 and 91% N_2), were used. Prior to testing, the ring-stage parasites were synchronized using sorbitol[19] and the suspension was adjusted for parasitemia and hematocrit, as described below. The infected red blood cells were distributed in 96-well microtiter plates (Corning, Santa Clara, CA, USA), 180 μL per well, to which 20 μL of different concentrations of the test drugs and controls had previously been added. The maximum concentration of 50 μg/mL (~157 μM) was tested at least three times for each compound against *P. falciparum* in blood cultures; drug activity was evaluated using an assay with commercially available monoclonal antibodies that had been raised against a parasite histidine and alanine-rich protein (HRP2) (MPFM ICLLAB-55A®, MPFG55P ICLLAB®, USA), as described[20]. The assay was performed using 0.5% parasitemia and 1.5% hematocrit, and the test quantification was read at 450 nm on a spectrophotometer (SpectraMax340PC^{384}, Molecular Devices).

The drug activity was expressed as the half-maximal inhibitory dose (IC_{50}) compared to the drug-free controls using the curve-fitting software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA)[21].

Cell cultures and cytotoxicity tests

The monkey kidney cell line (BGM) was cultured in 75 cm^2 sterile flasks containing RPMI 1640 medium that had been supplemented with 10% heat-inactivated fetal serum and 40 mg/L of gentamicin in a 5% CO_2 atmosphere at 37 °C. For the *in vitro* cytotoxicity experiments, the cell monolayer was trypsinized, washed with culture medium, distributed in a flat-bottomed 96 well plate (5×10^3 cells/well), and incubated for 18 hours at 37 °C for cell adherence.

For the cytotoxicity testing, we used the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, performed as previously described[22]. The BGM cell line was incubated with 20 µL of the compounds at different concentrations (200-25 µg/mL) for 24 hours in an atmosphere of 5% CO_2 at 37 °C.

For the MTT assay, which evaluates mitochondrial viability, 20 µL of MTT solution (5 mg/mL) were added, and the plates were incubated for an additional 3 hours. After incubation, the supernatants were carefully removed from the wells, followed by the addition of 100 µL of DMSO with thorough mixing. Optical densities at 570 and 630 nm (background) were determined using an ELISA reader.

Cell viability was expressed as the percentage of control absorbance obtained in untreated cells after subtracting the absorbance from the appropriate background. Finally, the minimum lethal dose for 50% of the cells (MLD_{50}) was determined as previously described[23] (**Figure 3**). The ratio between MLD_{50} and drug activity (IC_{50}) *in vitro* was used to determine the selectivity index (SI).

The synthesized 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinoline **3**-**13** derivatives with different substituents in the 4-positions of the 1*H*-1,2,3-triazole ring were tested against the W2-chloroquine-resistant *P. falciparum* clone.

Results and discussion

Chemistry

The synthetic route that was used to prepare the 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines (**3-13**) is shown in **Scheme 1**.

The commercially available compound 4,7-dichloroquinoline (**1**) was treated with sodium azide in refluxing MeOH to generate a 70% yield of the 4-azido-7-chloroquinoline (**2**) derivative after 24 hours. Compound **2** was characterized, and the data were in agreement with the literature[24]. The 1,3-dipolar cycloaddition reaction between the azide derivative (**2**) and the terminal alkynes was performed using Click chemistry reaction conditions, which consisted of sodium ascorbate, a Cu(I) catalyst, in H_2O/*t*-BuOH/THF (1:1:1) at 25 °C, to obtain 40-77% yields of only one 1,4-regioisomer of 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinoline derivatives **3-11**[25-27].

Azide group absorption was not observed in the IR spectrum of derivatives **3-10**. These compounds exhibited a chemical shift pattern for the quinoline ring. Both a

doublet in the region of 9.13 - 9.21 ppm, which corresponded to H2, and a doublet in the region of 7.80 - 7.95 ppm, which corresponded to H3, were observed, with a coupling constant (*J*) 4.6 Hz.

A double doublet, which corresponded to H6, was detected in the region of 7.78 - 7.81 ppm and had *J* = 9.0 Hz and *J* = 2.0 Hz, which corresponded to H5 and H8 coupling, respectively. In addition, triazole hydrogens are characteristic of these compounds and were observed in the region of 8.57 - 9.51 ppm as a singlet.

The quinolin-4-yl-1*H*-1,2,3-triazol alcohol (**10**) derivative was used as the raw material for the synthesis of compounds **11, 12** and **13**. Aldehyde **11** was prepared by the Swern oxidation of compound **10**, resulting in a 55% yield. OH group absorption was not observed in the FTIR spectrum of derivative **11**. The characteristic IR absorption representing carbonyl group stretching from the aldehyde at 1697 cm^{-1} were observed. The ^{1}H NMR spectra showed a singlet signal at 10.21 ppm, which corresponded to the aldehyde hydrogen. The ^{13}C NMR spectra also showed a signal at 185.0 ppm, which corresponded to the carbonyl carbon.

The fluorination of compound **10** with dimethylaminosulfur trifluoride (DAST) in CH_2Cl_2 at 25 °C produced a 70% yield of the monofluorinated derivative **12** after 24 hours. OH group absorption was not detected in the FTIR spectrum of derivative **12**, confirming the substitution of OH by fluorine. The ^{1}H NMR spectra revealed the presence of a doublet at 5.65 ppm with *J* = 48.48 Hz, which corresponded to the coupling of the methylene hydrogens to a fluorine atom. The ^{13}C NMR spectra revealed signals at 75.0 and 142.6 ppm with *J* = 159.8 and 20.0 Hz, respectively, which corresponded to the coupling of carbon to a fluorine atom. The ^{19}F NMR spectra revealed a characteristic signal at -204.22 ppm.

The aldehyde derivative **11** was treated with DAST using the same reaction conditions to generate a 60% yield of the difluorinated derivative **13**. The ^{1}H NMR spectra revealed a triplet at 7.41 ppm with *J* = 48.48 Hz, which indicated the coupling of hydrogen to fluorine atoms. The ^{13}C NMR spectra revealed signals at 110.0, 127.0 and 142.0 ppm, with *J* = 231.6, 3.14 and 27.4 Hz, respectively, which corresponded to the coupling of carbon to fluorine atoms. The ^{19}F NMR spectra revealed a characteristic signal at -112.56 ppm.

In vitro biological evaluation

The results of the anti-*P. falciparum* activity and cytotoxicity analyses of the 11 new compounds are summarized in **Table 1**. The compounds displayed IC_{50} values ranging from 1.4 ± 0.2 to 46± 2 μM in the anti-HPR2 assay. Compound **11** was the most active, with an IC_{50} = 1.4 ± 0.2 μM, and the least toxic, with the highest SI value (351). Compounds **3**, **5-7** and **13** were partially active, with IC_{50} values that were lower than 50 μM and SI values that were greater than 10, which are considered to be non-toxic. Compounds **4**, **8-10** and **12** were inactive. Taken together, the results of the *in vitro* anti-*P. falciparum* and toxicological activity analyses indicated that compounds **3**, **5-7, 11** and **13** can be considered to be prototypes for the development of new antimalarial drugs.

Conclusion

We have synthesized eleven new hybrid 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines **(3-13)** with different substituents at the 4-positions of the 1*H*-1,2,3-triazole ring. None of these compounds was toxic to HepG2 cells. Six compounds (**3**, **5-7**, **11** and **13**) exhibited anti-plasmodial activity against the W2 chloroquine-resistant *P. falciparum* clone, with IC_{50} values ranging from 1.4 to 46 μM.

Compound **11**, which contained an aldehyde group at the 4-position of 1*H*-1,2,3-triazol-1-yl, was the most active against *P. falciparum*, with IC_{50} values of 1.4 μM, and the least toxic, displaying the highest SI value (351). These data suggest that these compounds can be used as prototypes for other studies of compounds against *P. falciparum*.

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Conflict of Interest

All authors report no conflict of interest.

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Figure 1: Structures of quinoline and 1,2,3-triazole derivatives **(I-VI).**

Figure 2: Rational approach to the design of 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines **(3-13)**.

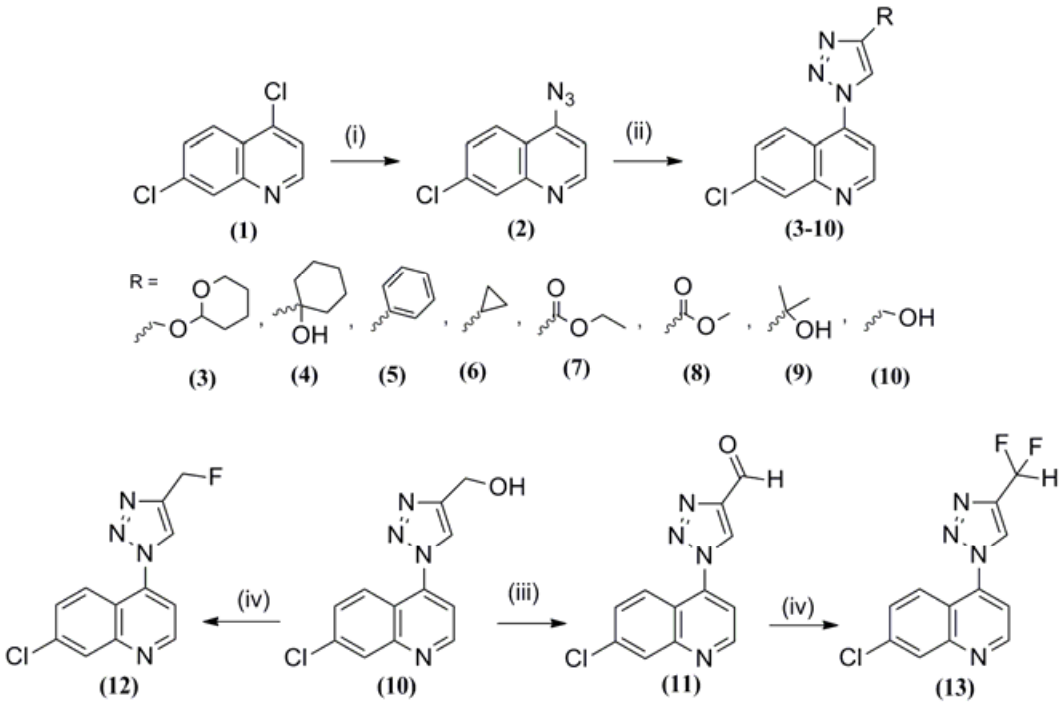
Figure 3: Dose response curve of actives and partial actives compounds compounds. Chloroquine that was the standard antimalarial used is also represented.

Scheme 1: Synthetic route used for the preparation of 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines **(3-10)**.

Table 1: Evaluation of the anti-plasmodial activity against a chloroquine-resistant W2 clone of *P. falciparum*, cytotoxicity against a human monkey kidney cell line (BGM) and drug selectivity index (SI) of compounds **3-13** and chloroquine.

Compounds	IC_{50} (µM)[a] *P. falciparum* Anti-HRP2	MDL_{50} BGM (µM)	SI MDL_{50} / IC_{50}
3	27 ± 5	≥ 581	22
4	83 ± 19	≥ 609	Inactive
5	17 ± 2	≥ 653	≥ 39
6	35 ± 15	≥ 738	21
7	14 ± 3	≥ 662	≥ 48
8	130 ± 6	≥ 694	Inactive
9	90 ± 18	≥ 694	Inactive
10	101 ± 25	≥ 777	Inactive
11	1.4 ± 0.2	477±22	351
12	109 ± 11	763	Inactive
13	46 ± 2	≥ 694	15
chloroquine	0.10 ± 0.077	490	4900

[a]IC_{50} <10 µM active; >10 and <50 µM partially active; >50 µM inactive.



Reagents and conditions: (i) NaN_3, MeOH, reflux, 24 h; (ii) appropriate acetylenes, L-ascorbic acid sodium salt, $CuSO_4.5H_2O$, H_2O/t-BuOH/THF (1:1:1), 25 °C, 24 h; (iii) ClCOCOCl, CH_2Cl_2, DMSO, TEA, -78 °C, 6 h; (iv) DAST, CH_2Cl_2, 25 °C, 24 h.