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Design, Synthesis and Antimalarial Activity of Second-Generation β-Carboline/Chloroquine Hybrids

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Abstract: As the resistance of Plasmodium to the existing antimalarials increases, there is a crucial need to expand the antimalarial drug pipeline. We recently identified potent antimalarial com pounds, namely harmiquins, hybrids derived from the β-carboline alkaloid harmine and 4-amino-7 chloroquinoline, a key structural motif of chloroquine (CQ). To further explore the structure−activity relationship, we synthesised 13 novel hybrid compounds at the position N-9 of the β-carboline ring and evaluated their efficacy in vitro against Plasmodium falciparum 3D7 and Dd2 strains (CQ sensi tive and multi-drug resistant, respectively). All compounds exhibit persistent antimalarial activity against both strains of P. falciparum. The most interesting derivatives had low nanomolar activity against both strains (IC50 (1) = 4.7 ± 1.3 nM against Pf3D7 and 6.5 ± 2.5 nM against PfDd2; IC50 (5) = 4.6 ± 0.6 nMagainst 3D7 and 10.5 ± 0.4 nMagainst Dd2). Resistance indices (RIs) ranged from 0.9 to 5.3 compared to CQ (RI = 14.4), highlighting their superior consistency in activity against both strains. The cytotoxicity screening performed on HepG2 revealed over 3 orders of magnitude higher IC50 for most of the compounds, with SIs from 711.0 to 8081.8. Spectroscopic studies explored the affinities of newly synthesised compounds for DNA, RNA, and HSA. Both tested hybrids, 2 and 7, were intrinsically fluorescent in an aqueous medium, characterised by remarkable Stokes shifts of emission maxima ($\Delta\lambda$ = +103 and +93 nm for 2 and 7, respectively). Fluorimetric experiments revealed that compound 2, with its shorter and more flexible linker, exhibited at least an order of magnitude higher affinity toward ds-DNAs versus ds-RNA and two orders of magnitude higher affinity toward GC-DNAs compared to 7. The behaviour of the investigated compounds upon binding to HSA is very similar, showing a strong hypsochromic shift of the emission maximum (almost $\Delta\lambda$ = −70nm)anddemonstrating their effectiveness as fluorimetric probes for distinguishing between DNA/RNA and proteins.

Keywords: β-carboline; chloroquine; hybrid; synthesis; malaria; fluorimetric probes.

Introduction : Malaria, a parasitic disease prevalent in tropical and subtropical regions, is caused by various Plasmodium species and is transmitted through the bite of infected female Anopheles mosquitoes. While six species of the Plasmodium parasite are known to infect humans, Plasmodium falciparum stands out for its capacity to produce substantial levels of blood stage parasites, contributing significantly to malaria-related fatalities, with African children under 5 years of age being the most vulnerable demographic group [1]. Despite the considerable progress in malaria control due to efforts made since 2000, a steady stagnation has been noted in the last decade. In 2022, a global estimate suggests there were approximately 249 million malaria cases worldwide, marking a rise of 5 million cases from the previous year. In 2015, which serves as the baseline year for the Global Technical

Strategy for Malaria 2016–2030 (GTS), an estimated 231 million malaria cases were reported [2]. The rise in malaria-related deaths in 2022, totalling 608,000 fatalities, from which more than 75% account for children under the age of 5, additionally under scores the persistent burden malaria imposes on affected populations. The impact of climate change is increasingly being acknowledged as a significant factor contributing to the distribution and transmission dynamics of malaria, together with disruptions in malaria prevention and treatment programs and overloaded healthcare systems during the COVID-19 pandemic [2–4]. Plasmodium resistance to antimalarials and mosquito resistance to insecticides are constantly on the rise. As current antimalarial treatment has liabilities and both emerging and well-documented resistance issues, there is a continued need to refill the development pipeline with new and improved candidates [5]. Intensive efforts in antimalarial research have resulted in the identification of numerous active compounds, which are currently progressing through various stages of drug development, ranging from preclinical studies to clinical trials. Our particular interests lie in the compounds derived from chloroquine (CQ), a 4-amino-7-chloroquinoline antimalarial drug, and β-carboline, due to their significant antimalarial activities. CQ has been a prominent antimalarial drug in use since the 1940s. However, similar to many other antimalarials, its effectiveness in therapy has been significantly corrupted by the emergence of resistant Plasmodium parasites [6,7]. Derivatisation of its quinoline core, which has been confirmed as crucial for antimalarial activity, with various scaffolds offers a strategy to combat resistance, as resistance appears to be specific to the chemical structure of CQ[8]. Thus, various attempts have been undertaken to address the resistance issues with CQandenhance its antimalarial efficacy by linking a wide range of structural motifs to the4 amino-7-chloroquine core [7]. Frequently, derivatisation of the 4-amino-7-chloroquine core yields compounds exhibiting notable in vitro activity against both sensitive and resistant P. falciparum strains, with IC50 values ranging from micromolar to low nanomolar levels, depending on the scaffold linked to the 4-amino-7-chloroquinoline. Additionally, certain compounds have demonstrated superior activity compared to CQ [7,9–19]. β-Carbolines are a group of diverse, both naturally derived and synthetic compounds that have been studied for their CNS-related, anti-inflammatory, antioxidant, and anticancer properties, highlighting their multifaceted biological activities [19,20]. The antimalarial activity of harmine, one of the most extensively investigated β-carboline that was first isolated from Peganum harmala seeds, has been well documented and has paved the way for the extensive research of synthetic and natural β-carbolines as potential antimalarial agents delivering not only novelty in chemical structures but also in mechanism of antimalarial activity [21–23]. Derivatisation of closely related tetrahydro-β-carbolines with CQ to obtain conjugates with antimalarial activity has recently been explored. These conjugates demonstrated moderate in vitro antimalarial activity against the CQ-resistant P. falciparum strain (W2) in the low IC50 micromolar range (IC50 from 0.49 to 9.28 μM) [24]. Our research group has been extensively exploring β-carboline hybrids as potential antimalarial compounds [25–31], and the most active ones were hybrids composed of β-carboline and CQ core. Poje et al. have studied the impact of various positions of substitution on the β-carboline on the antimalarial activity and have established that derivatives obtained by substitution at position 9 of the β-carboline are the most active compounds with IC50 values in the low nanomolar range (Figure 1) against CQ-sensitive (3D7) and resistant (Dd2) P. falciparum strains [28] Buildinguponthefavourableoutcomesofourpriorresearch[28],andrecognisingthe importance of the quinoline core for antimalarial activity, we have set to prepare two series he importance of the quinoline core for antimalarial activity, we have set to prepare two series of novel hybrid compounds, triazole-type (TT) and amide-type (AT) harmiquins of the 2nd generation. of the quinoline core for antimalarial activity, we have set to prepare two series of novel hybrid compounds.

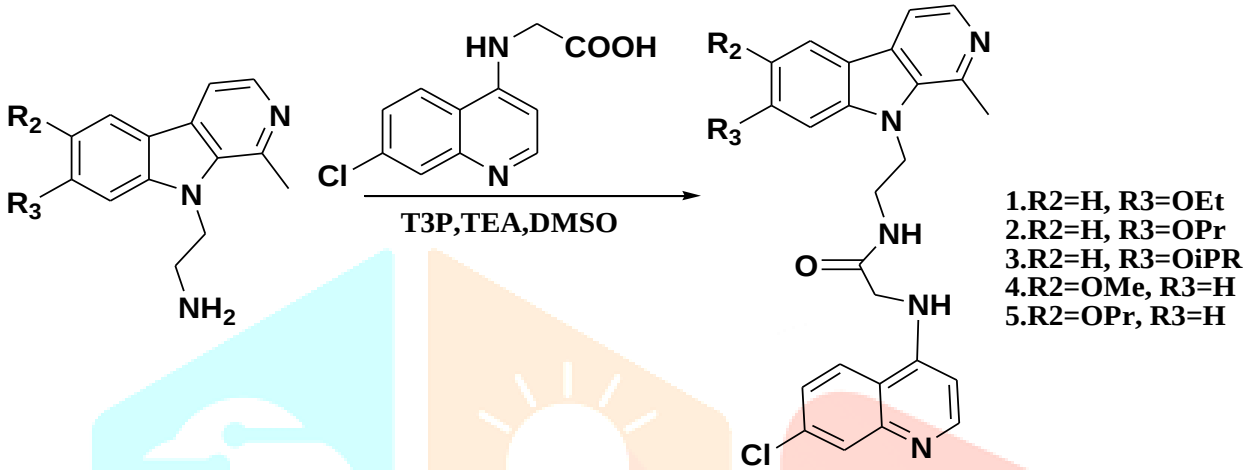
Results & discussion:

In this present work, we report the synthesis of the second generation of harmiquins, hybrid compounds composed of harmine/β-carboline and 4-amino-7-chloroquinoline moieties, covalently bound at the N-9 position of the β-carboline ring via anamide bond (AT harmiquins 1–5) or atriazole ring (TT harmiquins 6–10), differing in substituents at the C-1,O-6,andO-7positionsoftheβ-carboline ring.

General Procedure for the Synthesis of β-Carbolines :

To a solution of harmole 5 in anhydrous DMSO (5 mL), under an argon atmo sphere, Na2CO3 was added. The resulting suspension was stirred at room temperature for 10 min, followed by the addition of an appropriate alkyl bromide. The reaction mixture was stirred at room temperature, then at 40 ◦C or 60 ◦C, cooled down, poured into H2O (50 mL), and extracted with ethyl acetate (3 : 70 mL). The collected organic layers were washed with H2O and brine, dried over anhydrous sodium sulfate, filtered, and evapo rated under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH=8:1, then DCM:MeOH=75:25) and triturated with diethyl ether.

Reaction scheme:



General Procedure for the Synthesis of AT Harmiquins 1-5 A suspension of a 7-chloroquinoline-based carboxylic acid 4, amine 12–14, 22 or 24 (1.1 equivalents), and TEA (2 equivalents) in dry DMSO (1 mL) was stirred at room tem perature for 10 min, followed by the drop wise addition of T3P (≥50% in ethyl acetate, 1 equivalent). The reaction mixture was stirred at room temperature for 18 h. Afterwards, the reaction mixture was placed in an ultrasonic bath, and 5% NaOH was added dropwise until the formation of white precipitate was completed. The formed precipitate was filtered off and the crude product was purified by column chromatography (DCM/MeOH 85:15) and trituration with diethyl ether.

2-((7-Chloroquinolin-4-yl)amino)-N-(2-(7-ethoxy-1-methyl-9H-pyrido[3,4-b]indol-9 yl)ethyl)acetamide (1) Acid 4: 0.050 g, 0.21 mmol; amine 12: 0.063 g, 0.23 mmol; TEA: 0.043 g, 0.42 mmol; T3P: 0.067 g, 0.21 mmol; yield: 0.052 g (46%); mp 204–205 ◦C; IR (ATR, ν/cm−1) 3269, 2926, 1657, 1623, 1580, 1499, 1444, 1339, 1279, 1248, 1186, 1140, 1112, 1049, 975, 850, 804, 765, 732.

1H NMR (DMSO-d6) δ 8.38 (t, 1H, J = 5.5 Hz), 8.32 (d, 1H, J = 5.3 Hz), 8.26 (d, 1H, J = 9.0 Hz), 8.16 (d, 1H, J = 5.1 Hz), 8.09 (d, 1H, J = 8.5 Hz), 7.87 (d, 1H, J = 5.0 Hz), 7.82 (d, 1H, J = 2.3 Hz), 7.80 (d, 1H, J = 6.4 Hz), 7.50 (dd, 1H, J = 8.9, 2.3 Hz), 7.25 (s, 1H), 6.88 (d, 1H, J = 8.4 Hz), 6.10 (d, 1H, J = 5.3 Hz), 4.56 (t, 2H, J = 6.8 Hz), 4.15 (q, 2H, J = 6.8 Hz), 3.87 (d, 1H, J = 5.5 Hz), 3.51 (q, 1H, J = 6.9 Hz), 2.96 (s, 1H), 1.37 (t, 2H, J = 6.9 Hz); 13C NMR (DMSO-d6) δ 169.96, 160.30, 152.05, 150.79, 149.06, 143.38, 140.99, 138.21, 135.08, 134.09, 128.96, 127.70, 124.88, 124.71, 122.91, 117.95, 114.70, 112.72, 109.90, 99.45, 94.70, 63.95, 46.41, 43.74, 39.25, 23.50, 15.14; ESI-MS: m/z 488.20 (M + 1)+.

2-((7-Chloroquinolin-4-yl)amino)-N-(2-(1-methyl-7-propoxy-9H-pyrido[3,4-b]indol-9 yl)ethyl)acetamide (2) Acid 4: 0.050 g, 0.21 mmol; amine 13: 0.066 g, 0.23 mmol; TEA: 0.043 g, 0.42 mmol; T3P: 0.067 g, 0.21 mmol; yield: 0.021 g (20%); mp 232.5–234.5 ◦C; IR (ATR, ν/cm−1) 2963, 1656, 1623, 1581, 1444, 1340, 1278, 1248, 1188, 1140, 1048, 988, 851, 798, 637; 1H NMR (DMSO-d6) δ 8.38 (t, 1H, J = 6.1 Hz), 8.33 (d, 1H, J = 5.4 Hz), 8.25 (d, 1H, J = 9.0 Hz), 8.16 (d, 1H, J = 5.2 Hz), 8.08 (d, 1H, J = 8.6 Hz), 7.86 (d, 1H, J = 5.2 Hz), 7.82 (d, 1H, J = 2.3 Hz), 7.79 (t, 1H, J = 6.1 Hz), 7.50 (dd, 1H, J = 9.0, 2.3 Hz), 7.26 (d, 1H, J = 2.1 Hz), 6.88 (dd, 1H, J = 8.6, 2.1 Hz), 6.12 (d, 1H, J = 5.4 Hz), 4.83–4.77 (m, 1H), 4.55 (t, 2H, J = 7.2 Hz), 3.88 (d, 2H, J = 5.9 Hz), 3.49 (q, 2H, J = 7.2 Hz), 2.96 (s, 3H), 1.32 (d, 6H, J = 6.0 Hz); 13C NMR (DMSO-d6) δ 169.97, 159.15, 152.15, 150.75, 149.17, 143.37,

140.99, 138.23, 135.14, 134.05, 128.92, 127.79, 124.87, 124.69, 122.98, 117.98, 114.74, 112.72, 110.61, 99.48, 96.17, 70.08, 46.39, 43.73, 39.15, 23.52, 22.39; ESI-MS: m/z 502.2 (M + 1)+.

2-((7-Chloroquinolin-4-yl)amino)-N-(2-(7-isopropoxy-1-methyl-9H-pyrido[3,4-b]indol-9 yl)ethyl)acetamide (3) Acid 4: 0.028 g, 0.12 mmol; amine 14: 0.037 g, 0.13 mmol; TEA: 0.024 g, 0.24 mmol; T3P: 0.038 g, 0.12 mmol; yield: 0.022 g (37%); mp 249.5–255.5 ◦C; IR (ATR, ν/cm−1) 3384, 3198, 2975, 1684, 1624, 1586, 1526, 1449, 1388, 1334, 1281, 1232, 1198, 1114, 1081, 1025, 986, 935, 857, 821, 810, 759, 739, 643; 1H NMR (DMSO-d6) δ 8.38 (t, 1H, J = 6.1 Hz), 8.33 (d, 1H, J = 5.4 Hz), 8.25 (d, 1H, J = 9.0 Hz), 8.16 (d, 1H, J = 5.2 Hz), 8.08 (d, 1H, J = 8.6 Hz), 7.86 (d, 1H, J = 5.2 Hz), 7.82 (d, 1H, J = 2.3 Hz), 7.79 (t, 1H, J = 6.1 Hz), 7.50 (dd, 1H, J = 9.0, 2.3 Hz), 7.26 (d, 1H, J = 2.1 Hz), 6.88 (dd, 1H, J = 8.6, 2.1 Hz), 6.12 (d, 1H, J = 5.4 Hz), 4.83–4.77 (m, 1H), 4.55 (t, 2H, J = 7.2 Hz), 3.88 (d, 2H, J = 5.9 Hz), 3.49 (q, 2H, J = 7.2 Hz), 2.96 (s, 3H), 1.32 (d, 6H, J = 6.0 Hz); 13C NMR (DMSO-d6) δ 169.97, 159.15, 152.15, 150.75, 149.17, 143.37, 140.99, 138.23, 135.14, 134.05, 128.92, 127.79, 124.87, 124.69, 122.98, 117.98, 114.74, 112.72, 110.61, 99.48, 96.17, 70.08, 46.39, 43.73, 39.15, 23.52, 22.39; ESI-MS: m/z 502.2 (M + 1)+.

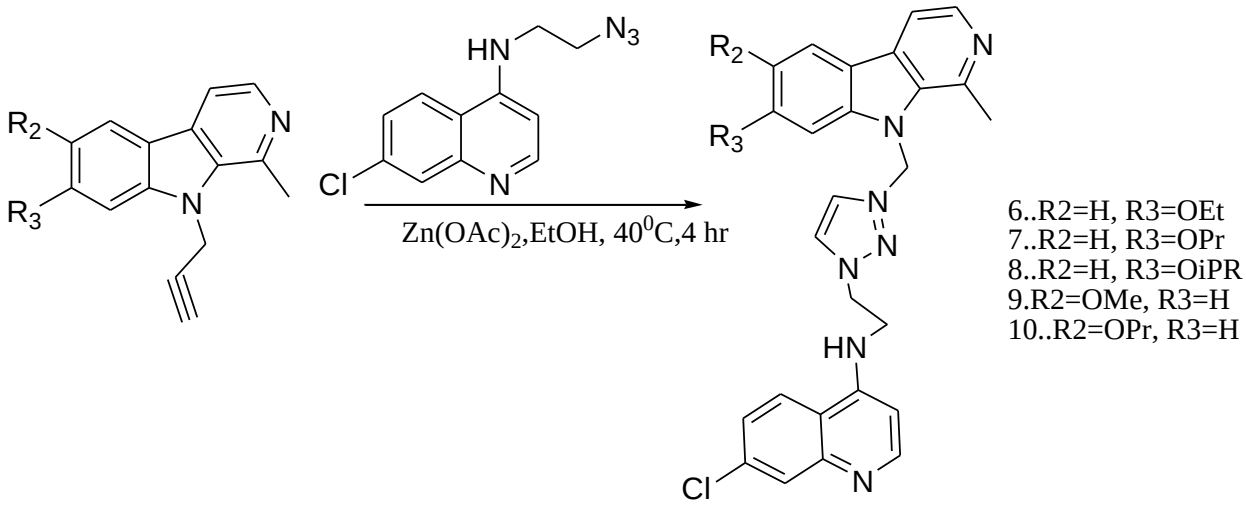
2-((7-Chloroquinolin-4-yl)amino)-N-(2-(6-methoxy-1-methyl-9H-pyrido[3,4-b]indol-9 yl)ethyl)acetamide (4) Acid 4: 0.046 g, 0.20 mmol; amine 24: 0.055 g, 0.22 mmol; TEA: 0.040 g, 0.40 mmol; T3P: 0.062 g, 0.20 mmol; yield: 0.037 g (40%); mp 219.5–222.5 ◦C; IR (ATR, ν/cm−1) 3272, 2920, 1666, 1611, 1489, 1408, 1374, 1226, 1200, 1178, 1126, 1048, 1022, 983, 899, 850, 806, 703, 670, 619; 1H NMR (DMSO-d6) δ 8.35 (d, 1H, J = 6.0 Hz), 8.33 (d, 1H, J = 5.5 Hz), 8.25 (d, 1H, J = 9.0 Hz), 8.18 (d, 1H, J = 5.2 Hz), 7.98 (d, 1H, J = 5.2 Hz), 7.82 (d, 1H, J = 2.3 Hz), 7.80 (d, 1H, J = 2.7 Hz), 7.65 (d, 1H, J = 9.0 Hz), 7.50 (dd, 1H, J = 9.0, 2.3 Hz), 7.23 (dd, 1H, J = 9.0, 2.5 Hz), 6.09 (d, 1H, J = 5.5 Hz), 4.60 (t, 2H, J = 7.0 Hz), 3.87 (s, 3H), 3.85 (d, 2H, J = 5.9 Hz), 3.48 (q, 2H, J = 6.6 Hz), 2.97 (s, 3H); 13C NMR (DMSO-d6) δ 169.84, 154.15, 151.97, 150.83, 148.98, 141.87, 137.46, 136.67, 135.37, 134.12, 128.34, 127.63, 124.90, 124.71, 121.45, 118.40, 117.93, 113.53, 111.61, 103.97, 99.46, 56.15, 46.29, 43.92, 40.41, 23.64; ESI-MS: m/z 474.30 (M + 1)+.

2-((7-Chloroquinolin-4-yl)amino)-N-(2-(1-methyl-6-propoxy-9H-pyrido[3,4-b]indol-9 yl)ethyl)acetamide (5) Acid 4: 0.049 g, 0.21 mmol; amine 22: 0.065 g, 0.23 mmol; TEA: 0.042 g, 0.42 mmol; T3P: 0.066 g, 0.21 mmol; yield: 0.017 g (16%); mp 238.5–245.5 ◦C; IR (ATR, ν/cm−1) 3273, Molecules 2024, 29, 5991 23 of 3 2964, 1656, 1611, 1582, 1488, 1450, 1374, 1225, 1196, 991, 850, 802, 766, 618; 1H NMR (DMSO d6) δ 8.33 (d, 2H, J = 4.7 Hz), 8.24 (d, 1H, J = 9.0 Hz), 8.17 (d, 1H, J = 5.2 Hz), 7.97 (d, 1H, J = 5.1 Hz), 7.81 (d, 1H, J = 1.9 Hz), 7.79 (d, 1H, J = 2.2 Hz), 7.73 (t, 1H, J = 5.7 Hz), 7.63 (d, 1H, J = 9.0 Hz), 7.49 (dd, 1H, J = 9.0, 2.0 Hz), 7.23 (dd, 1H, J = 8.9, 2.3 Hz), 6.08 (d, 1H, J = 5.4 Hz), 4.59 (t, 2H, J = 6.9 Hz), 4.03 (t, 2H, J = 6.5 Hz), 3.84 (d, 2H, J = 5.8 Hz), 3.48 (q, 3H, J = 6.5 Hz), 2.97 (s, 3H), 1.79 (h, 2H, J = 7.1 Hz), 1.03 (t, 3H, J = 7.4 Hz); 13C NMR (DMSO-d6) δ 169.91, 153.50, 152.25, 150.65, 149.30, 141.84, 137.50, 136.65, 135.38, 134.00, 128.35, 127.89, 124.83, 124.66, 121.50, 118.75, 117.99, 113.55, 111.55, 104.89, 99.47, 70.17, 46.31, 43.91, 40.42, 23.66, 22.71, 11.01; ESI-MS: m/z 502.20 (M + 1)+. 3.1.8.

General Procedure for the Synthesis of TT Harmiquins 6–10.

To a solution of an alkyne and the 7-chloroquinoline-based azide 3 (1.1 equivalents) in EtOH (5mL), acatalytic amount of $Zn(OAc)_2$ was added. The reaction mixture was stirred at 40 ^{0} C for 4 h. Upon completion of the reaction, the solvent was removed under reduced pressure. The residue was purified by column chromatography (with an additional Al_2O_3 layer in order to remove Zn salts) with EtOH:Pet ether=7:3 as a mobile phase. The crude product was triturated with diethyl ether or MeOH (compounds 44 and 45).

Reaction scheme:



6..R2=H, R3=OEt
7..R2=H, R3=OPr
8..R2=H, R3=OiPR
9.R2=OMe, R3=H
10..R2=OPr, R3=H

7-Chloro-N-(2-(4-((7-ethoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3 triazol-1-yl)ethyl)quinolin-4-amine (6) Alkyne 15: 0.060 g, 0.23 mmol; azide 3: 0.062 g, 0.25 mmol; yield: 0.067 g (58%); mp 228–230 ◦C;

IR (ATR, ν/cm−1) 2972, 1622, 1579, 1444, 1408, 1324, 1250, 1181, 1140, 1047, 954, 910, 876, 814, 765, 641; 1H NMR (DMSO-d6) δ 8.31 (d, 1H, J = 5.4 Hz), 8.16 (d, 1H, J = 5.2 Hz), 8.06 (d, 1H, J = 8.6 Hz), 8.03 (d, 1H, J = 9.1 Hz), 7.97 (s, 1H), 7.86 (d, 1H, J = 5.2 Hz), 7.79 (d, 1H, J = 2.2 Hz), 7.39 (dd, 1H, J = 9.0, 2.2 Hz), 7.37 (d, 1H, J = 6.0 Hz), 7.24 (d, 1H, J = 2.1 Hz), 6.85 (dd, 1H, J = 8.6, 2.1 Hz), 6.45 (d, 1H, J = 5.4 Hz), 5.82 (s, 2H), 4.57 (t, 2H, J = 6.0 Hz), 4.08 (q, 2H, J = 7.0 Hz), 3.69 (q, 2H, J = 5.8 Hz), 2.98 (s, 3H), 1.35 (t, 3H, J = 6.9 Hz); 13C NMR (DMSO-d6) δ 159.74, 151.59, 149.70, 148.70, 143.85, 142.62, 141.00, 137.92, 134.56, 133.52, 128.48, 127.30, 124.31, 123.72, 123.41, 122.29, 117.29, 114.27, 112.17, 109.60, 98.74, 94.43, 63.42, 47.84, 42.32, 23.07, 14.58; ESI-MS: m/z 512.20 (M + 1)+.

7-Chloro-N-(2-(4-((1-methyl-7-propoxy-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3 triazol-1-yl)ethyl)quinolin-4-amine (7) Alkyne 16: 0.060 g, 0.22 mmol; azide 3: 0.059 g, 0.24 mmol; yield: 0.082 g (72%); mp 246–250 ◦C; IR (ATR, ν/cm−1) 3204, 2964, 1622, 1578, 1498, 1445, 1429, 1366, 1341, 1323, 1181, 1140, 1081, 1048, 982, 935, 910, 877, 815, 766, 732, 640; 1H NMR (DMSO-d6) δ 8.31 (d, 1H, J = 5.4 Hz), 8.17 (d, 1H, J = 5.2 Hz), 8.07 (d, 1H, J = 8.6 Hz), 8.03 (d, 1H, J = 9.1 Hz), 7.96 (s, 1H), 7.86 (d, 1H, J = 5.2 Hz), 7.78 (d, 1H, J = 2.1 Hz), 7.39 (dd, 1H, J = 9.0, 2.2 Hz), 7.36 (d, 1H, J = 5.6 Hz), 7.25 (d, 1H, J = 1.8 Hz), 6.87 (dd, 1H, J = 8.6, 2.0 Hz), 6.45 (d, 1H, J = 5.5 Hz), 5.83 (s, 2H), 4.57 (t, 2H, J = 6.0 Hz), 3.99 (t, 2H, J = 6.6 Hz), 3.69 (q, 2H, J = 5.8 Hz), 2.97 (s, 3H), 1.76 (h, 2H, J = 7.1 Hz), 0.99 (t, 3H, J = 7.4 Hz); 13C NMR (DMSO-d6) δ 159.33, 151.06, 149.10, 148.17, 143.36, 142.07, 140.42, 137.36, 134.00, 132.93, 127.92, 126.77, 123.72, 123.14, 122.78, 121.71, 116.73, 113.70, 111.60, 109.05, 98.17, 93.89, 68.73, 47.27, 41.75, 22.49, 21.47, 9.87; ESI-MS: m/z 526.15 (M + 1)+.

7-Chloro-N-(2-(4-((7-isopropoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3 triazol-1-yl)ethyl)quinolin-4-amine (8) Alkyne 17: 0.060 g, 0.22 mmol; azide 3: 0.059 g, 0.24 mmol; yield: 0.054 g (47%); mp 234–236 ◦C; IR (ATR, ν/cm−1) 2974, 1622, 1578, 1445, 1408, 1324, 1182, 1139, 1111, 1047, 909, 875, 803; 1H NMR (DMSO-d6) δ 8.31 (d, J = 5.4 Hz, 1H), 8.16 (d, J = 5.2 Hz, 1H), 8.06 (d, J = 4.8 Hz, 1H),8.05 (d, J =5.1 Hz, 1H),7.97 (s, 1H), 7.86 (d, J = 5.1 Hz, 1H), 7.79 (d, J = 1.7 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 7.25 (d, J = 1.9 Hz, 1H), 5.82–5.80 (m, 2H), 6.84 (dd, J = 8.6, 2.0 Hz, 1H), 6.45 (d, J = 5.3 Hz, 1H), 5.81 (s, 2H), 4.75 (h, J = 6.0 Hz, 1H), 4.57 (t, J = 6.0 Hz, 2H), 3.70 (q, J = 5.8 Hz, 2H), 2.96 (s, 3H), 1.28 (d, J = 6.0 Hz, 6H); 13C NMR (DMSO-d6) δ 158.58, 151.38, 149.84, 148.46, 143.94, 142.72, 140.90, 137.85, 134.60, 133.63, 128.52, 127.10.

7-Chloro-N-(2-(4-((6-methoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3 triazol-1-yl)ethyl)quinolin-4-amine (9) Alkyne 25: 0.030 g, 0.12 mmol; azide 3: 0.033 g, 0.13 mmol; yield: 0.028 g (46%); mp 250. ◦C; IR (ATR, ν/cm−1) 3143, 3061, 2958, 1611, 1579, 1487, 1448, 1431, 1402, 1370,

1324, 1225, 1195, 1140, 1083, 1083, 1046, 1022, 980, 909, 876, 818, 806, 734, 725, 690, 609; 1H NMR(DMSO-d6) δ 8.31 (d, 1H, J = 5.4 Hz), 8.18 (d, 1H, J = 5.2 Hz), 8.04 (d, 1H, J = 9.1 Hz), 7.97 (d, 1H, J = 5.2 Hz), 7.93 (s, 1H), 7.79 (d, 1H, J = 2.2 Hz), 7.77 (d, 1H, J = 2.5 Hz), 7.64 (d, 1H, J = 9.0 Hz), 7.42 (dd, 1H, J = 9.0, 2.2 Hz), 7.40 (d, 1H, J = 5.5 Hz), 7.14 (dd, 1H, J = 9.0, 2.5 Hz), 6.44 (d, 1H, J = 5.5 Hz), 5.82 (s, 2H), 4.55 (t, 2H, J = 6.0 Hz), 3.86 (s, 3H), 3.69 (q, 2H, J = 5.9 Hz), 2.99 (s, 3H); 13C NMR (DMSO-d6) δ 154.25, 152.03, 150.24, 149.13, 144.47, 142.30, 137.69, 136.38, 135.39, 134.08, 128.37, 127.74, 124.86, 124.32, 123.86, 121.64, 118.27, 117.79, 113.50, 111.98, 103.96, 99.22, 56.14, 48.35, 42.76, 23.68; ESI-MS: m/z 498.20 (M + 1)+.

7-Chloro-N-(2-(4-((1-methyl-6-propoxy-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3 triazol-1-yl)ethyl)quinolin-4-amine (10) Alkyne 26: 0.045 g, 0.16 mmol; azide 3: 0.044 g, 0.18 mmol; yield: 0.034 g (40%); mp 205–206 ◦C; IR (ATR, ν/cm−1) 3207, 2969, 1610, 1577, 1488, 1448, 1404, 1281, 1241, 1194, 1139, 1047, 990, 908, 880, 820, 807, 666, 639, 622; 1H NMR (DMSO-d6) δ 8.31 (d, J = 5.4 Hz, 1H), 8.17 (d, J = 5.2 Hz, 1H), 8.04 (d, J = 9.1 Hz, 1H), 7.96 (d, J = 5.2 Hz, 1H), 7.93 (s, 1H), 7.79 (d, J = 2.2 Hz, 1H), 7.77 (d, J = 2.5 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.43 (dd, J = 9.0, 2.2 Hz, 1H), 7.40 (d, J = 5.7 Hz, 1H), 7.14 (dd, J = 8.9, 2.5 Hz, 1H), 6.44 (d, J = 5.5 Hz, 1H), 5.81 (s, 2H), 4.55 (t, J = 6.0 Hz, 2H), 4.02 (t, J = 6.5 Hz, 2H), 3.69 (q, J = 5.9 Hz, 3H), 2.99 (s, 3H), 1.79 (h, J = 7.3 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H); 13C NMR (DMSO-d6) δ 153.61, 150.26, 149.11, 144.47, 142.25, 137.68, 136.35, 135.40, 134.09, 128.40, 127.73, 124.86, 124.33, 123.87, 121.69, 118.64, 117.79, 113.53, 111.91, 104.91, 99.22, 70.17, 48.37, 42.77, 23.67, 22.71, 11.01; ESI-MS: m/z 526.20 (M + 1)+.

Biological activitry:

In Vitro Antiplasmodial Activity: The activity of the title compound against the erythrocytic stage of the Plasmodium lifecycle in vitro was investigated by using two P. falciparum strains, aCQ-sensitive (Pf3D7)and a multi-drug resistant (PfDd2)(Table3),by employing previously described methods[8–10].The resistance index (RI),which represents a ratio between IC50 obtained against PfDd2andPf3D7,was also calculated (Table below). In vitro antiplasmodial activity of harmiquins 1–10 against P.falciparum erythrocytic stage (Pf3D7andPfDd2strains)and resistance index(RI)calculated as the ratio between multi-drug resistant(PfDd2)and CQ-sensitive (Pf3D7)P.falciparum strains.

Compound	LV	VV	HA	RB	HBA	HBD	TPSA
1	0	0	34	8	5	1	82
2	1	0	35	9	4	2	80
3	1	0	36	6	5	2	79
4	1	0	33	9	5	2	84
5	0	0	37	9	4	1	80
6	1	0	35	7	5	2	83
7	1	0	38	8	5	2	83
8	0	0	39	9	5	2	84
9	0	0	40	8	5	2	85
10	1	0	39	9	5	2	83

Conclusions: In this paper, we present the synthesis of a novel series of β-carboline/7 chloroquinoline hybrids 1–10, evaluation of their in vitro antiplasmodial activity against sensitive and multi-drug-resistant Dd2 P. falciparum strains, and cytotoxicity against the human cell line HepG2. Their antiplasmodial activity against the erythro cytic stage of P. falciparum demonstrated strong effectiveness against both CQ-sensitive and resistant Plasmodium strains.

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